preci

Product Guide

ADMET

2025

preci

Syllabus

1. Introduction

ADME strategies continue to evolve alongside the emergence of new therapeutic modalities. At Preci, our efforts in the ADMET field focus on systematizing available in vitro approaches and delivering a comprehensive toolbox for permeability, distribution, metabolism, excretion, and toxicity studies. We stay aligned with the ongoing progress in the field—offering a broad product portfolio that spans from classical assay systems, such as human hepatocytes and microsomes, to models tailored for novel chemical entities.

Particular attention is dedicated to developing ADME solutions for peptides and biologics.
Most of the assay systems we offer are based on human-derived materials. Through years of experience, we have developed rigorous and ethical approaches to working with human tissues—ensuring full compliance with global bioethical standards and consistent performance in real-world assays. Our quality assurance process directly aligns product release testing with commonly used assay validation workflows, minimizing discrepancies between scientists' expectations and our quality control data.

Additionally, we are always open to running a new assay or reference compounds set validating our product for the specific demand. This brochure attempts to summarize all technical data about our products and survey through our capabilities devoted to in vitro ADMET scientists.

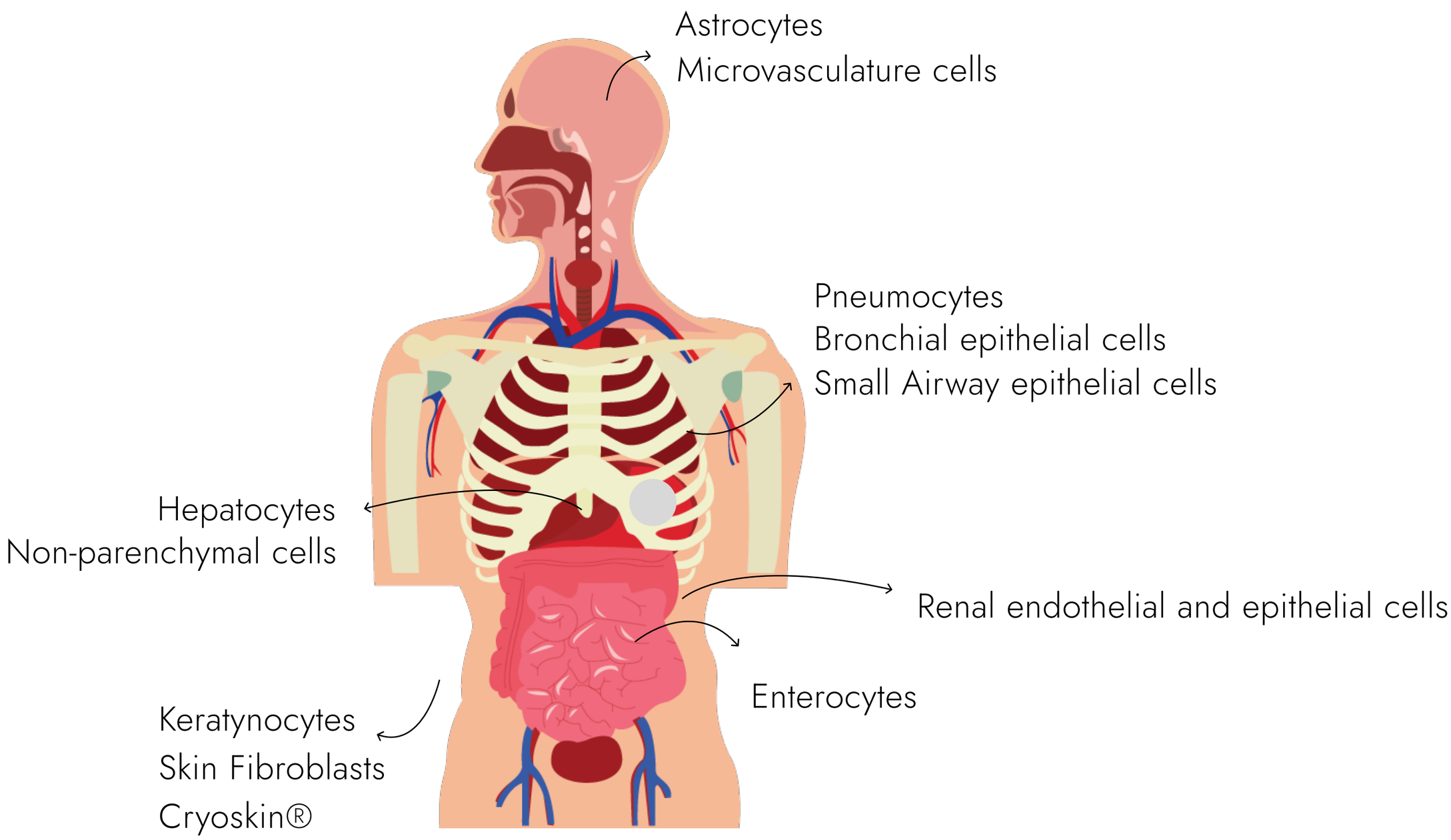


Figure 1. Cellular products provided by Preci

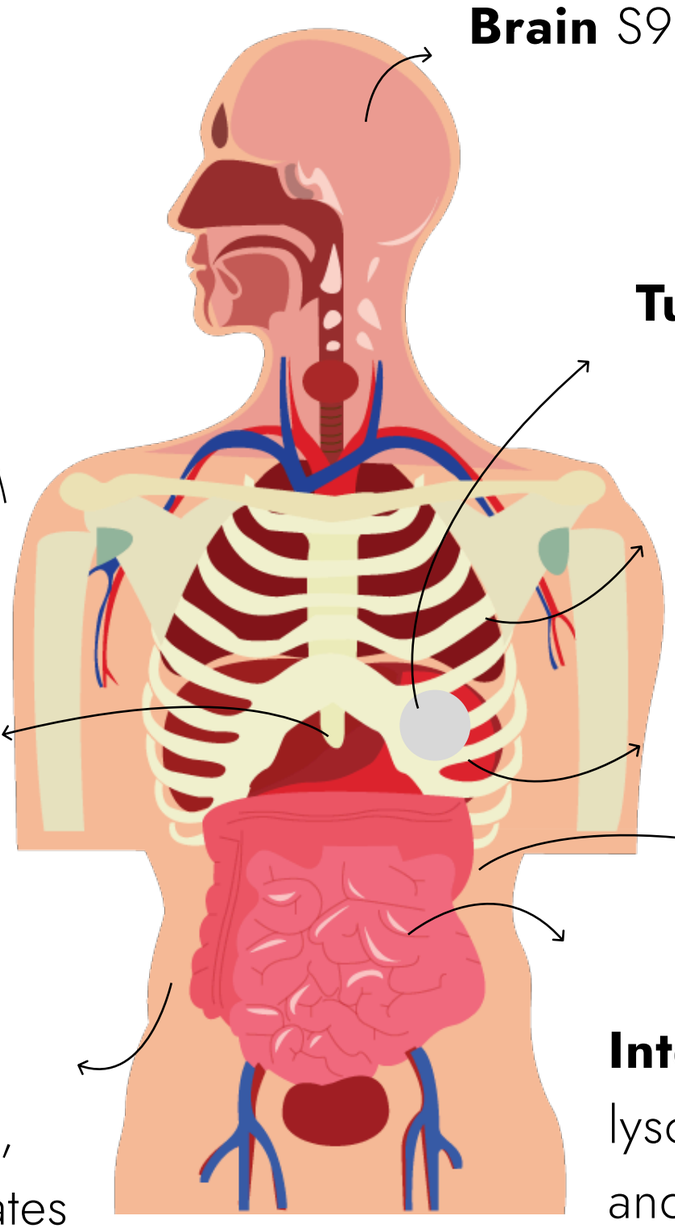


Figure 2. Subcellular products provided by Preci

Within our product portfolio, two distinct classes can be defined: **cellular** models and **subcellular** models. The cellular core encompasses complex human-derived systems capable of remaining viable—and in some cases proliferative—in vitro. These models are carefully engineered to recapitulate the physiological route of a drug through the human body. However, designing a mechanistically definitive assay solely with cellular systems can often be challenging due to their inherent biological complexity.

Subcellular models therefore provide the biochemical counterpart to this toolkit, offering a more streamlined way to dissect drug metabolism and disposition. They enable the study of uptake, binding, metabolism, and efflux as independent processes. All subcellular products are generated through controlled cell fractionation and rigorously evaluated to ensure consistent performance in the most widely used applications.

We continuously refine and expand our technologies, which has led to the introduction of next-generation products such as extended-viability human hepatocytes (EVH), enterocytes, and Cryoskin® systems. These additions enhance our ability to model drug metabolism, off-target effects, and tissue-specific liabilities with greater precision.

This brochure reviews the products by their intended application, for easier navigation, and intend-focused review. Please, check our website for any updates or more products.

2. Small molecules metabolism product portfolio

This chapter surveys through cellular models, subcellular fractions, and plasma products available to the researchers studying small molecules.

2.1. Hepatic metabolism

The main goals of in vitro ADME experimentation during the investigation of new molecular entities are to evaluate the molecule's stability against drug-metabolizing enzymes and to identify the structural nature of the metabolites formed. The liver exhibits the highest activity of drug-metabolizing enzymes toward small molecules and therefore represents the primary organ for metabolic stability assessment.

Phase I drug-metabolizing enzymes include cytochrome P450s (CYPs), flavin-containing monooxygenases, and various oxidases such as alcohol dehydrogenases. These intracellular enzymes are responsible for oxidative transformations of xenobiotics, with the CYP family accounting for the metabolism of most small-molecule drugs. Phase II enzymes, primarily transferases, catalyze the conjugation of reactive intermediates within cells and are equally important for estimating overall drug metabolism rates and for metabolite profiling. It is well-known that while human liver microsomes are perfectly suited model for Phase I metabolism of small molecules with high clearance, their applicability to slowly metabolized compounds and Phase II metabolism is limited. To encompass the whole amount of applications we offer the portfolio of liver subcellular fractions and cells.

For compounds with high hepatic extraction ratios (EH > 70%), conventional in vitro systems such as suspension-culture hepatocytes and liver microsomes provide reliable results. However, low-clearance compounds (EH < 30%) require more advanced models with prolonged metabolic competence, including plateable hepatocytes, hepatocyte spheroids, or Preci's EVH system—an extended-viability suspension hepatocyte culture designed for long-term stability and accurate clearance assessment.

	High clearance, EH>70%	Medium clearance	Low clearance, EH<30%
Human Liver Microsomes	+++	+	-
Suspension PHH*	+++	++	-
Plateable PHH*	+	+	++
EVH**	+++	+++	+++
Spheroid PHH*	+	+	+++

* pooled human hepatocytes
* extended viability pooled human hepatocytes

Table 1. Summarizing the applicability of various assay systems towards the metabolic profiling of the high, medium, and low-clearance compounds.

Pooled Human Liver Microsomes (MIC-H50, MIC-H100)

Pooled Human Liver Microsomes are truly working horses of the metabolic stability screening during small molecule drug discovery. They are simple to use in the routine biochemical workflow, batch-to-batch stable, and predictive in Phase I metabolic stability. We provide Pooled Human Liver Microsomes as pool-50 (MIC-H50) and pool-100 (MIC-H100) versions. Both versions have low (<20%) batch-to-batch deviation in metabolic stability of the tested substrates. Preci has reduced the cost of the production to guarantee the market best cost/assay value for your studies.

Feature	Specification value, min	Specification value, max
General		
BCA-based protein concentration, mg/mL	20	22
Volume/vial, mL	0.5	0.5
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Total CYP, nmole/mg	0.25	
Metabolic parameters		
Verapamil, Clint (uL/min/mg), 3A4	250	500
Testosterone, Clint (uL/min/mg), 3A4	60	350
Diclofenac, Clint (uL/min/mg), 2C9	180	120
Phenacetin, Clint (uL/min/mg), 1A2	20	360
Imipramine, Clint (uL/min/mg), 2C19	15	40
Benzydimine, Clint (uL/min/mg), FMO	10	35
Metoprolol, Clint (uL/min/mg), 2D6	5	15

Pooled Human Liver S9 fractions (S9-H50)

Pooled Human Liver S9 is a well-characterized instrument for studying both Phase I and Phase II compound stability. They still lack essential cellular machinery to fully simulate the compound faith in hepatic tissue, however, are good in getting the general understanding of the compound metabolic pathways. We deliver that product as a pool of 50 donors in a single vial.

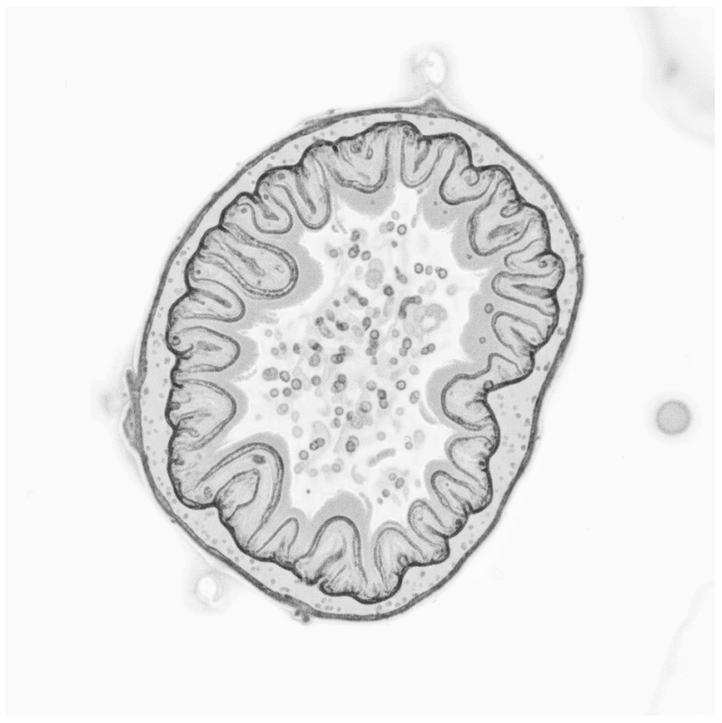
Feature	Specification value, min	Specification value, max
General		
BCA-based protein concentration, mg/mL	20	22
Volume/vial, mL	1	1
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Metabolic parameters		
Verapamil, Clint (uL/min/mg), 3A4	60	110

Pooled Human Liver Homogenate (HMG-LIN)

Pooled Human Liver Homogenate is a complex matrix, prepared by homogenizing the liver tissue in the buffered medium. It contains the major drug-metabolizing enzymes, as well as hydrolases, so can be used in investigation of hydrolytically-unstable groups.

Feature	Specification value, min
General	
Dilution factor	1:3
Volume/vial, mL	1
HBV, via qPCR	Negative
HCV, via qPCR	Negative
HIV, via qPCR	Negative
Metabolic parameters	
Verapamil, Clint (uL/min/mg), 3A4	60

Pooled Human Liver Mitochondria (MIT-LIN)

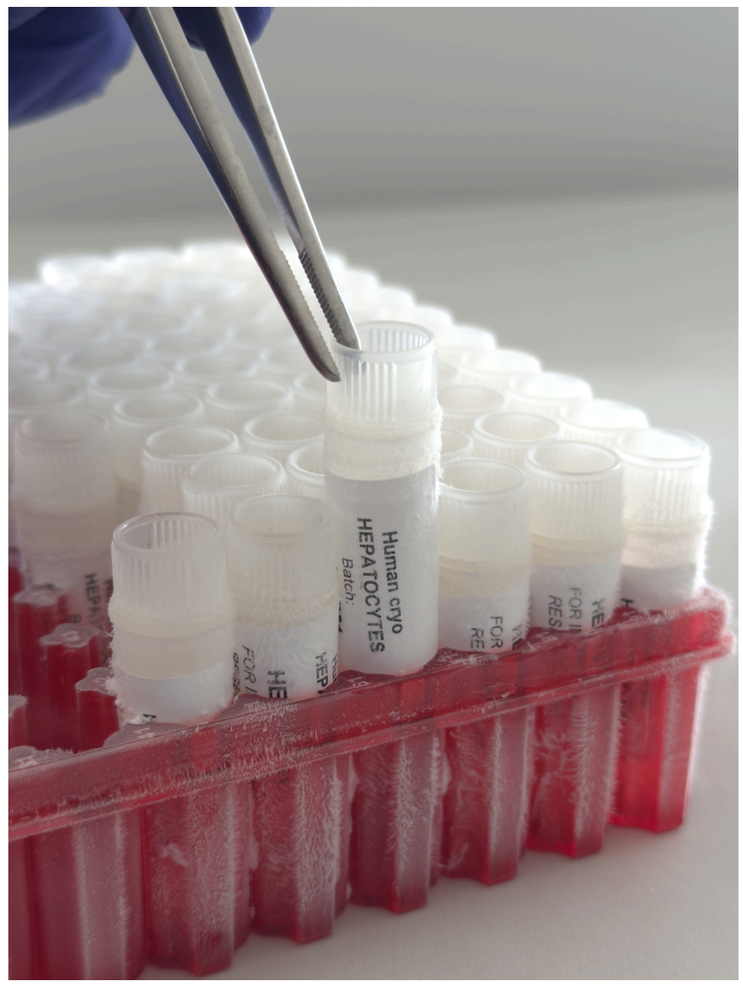


Pooled Human Liver Mitochondria is a well-characterized drug metabolism model for primary and secondary amines, which are usually unstable towards MAO metabolism. Every batch of our human liver mitochondria is pre-characterized for amine oxidation via MAO-A and MAO-B enzymes.

Feature	Specification value, min	Specification value, max
General		
BCA-based protein concentration, mg/mL	20	22
Volume/vial, mL	0,5	0,55
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Metabolic parameters		
MAO-A and MAO-B activity	confirmed	confirmed

Pooled Suspension Human Hepatocytes (HEP-S-10, HEP-S-20)

Pooled Suspension Human Hepatocytes is highly potent cellular assay system for metabolic stability of compounds in vitro, as well as metabolite identification and mechanistic studies. Unlike subcellular tools, pooled suspension human hepatocytes offer integration of uptake transporters, viable intracellular machinery, and naturally occurring enzyme levels. Our human hepatocytes products offer unmatched low cost/assay value, as well as highly stable and IVIVE-relevant levels of drug metabolizing enzymes. Additionally, per request those products can be validated on the customer-provided testing set of compounds for better integration into the existing testing workflows.



Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial	6	8
Viability upon thawing	80	100
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Metabolic parameters		
7-OH Coumarin, Clint (uL/min/mln cells), phase II	75	
Verapamil, Clint (uL/min/mln cells), 3A4	46	80
Testosterone, Clint (uL/min/mln cells), 3A4	100	200
Diclofenac, Clint (uL/min/mln cells), 2C9	50	100
Phenacetin, Clint (uL/min/mln cells), 1A2	5	10
Imipramine, Clint (uL/min/mln cells), 2C19	7	15
Propornolol, Clint (uL/min/mln cells), FMO	3	10
Metoprolol, Clint (uL/min/mln cells), 2D6	2	5
Midazolam,Clint (uL/min/mln cells), 3A4	25	45

Animal Liver-Derived Products

Animal products, provided in collaboration with **MileCell Bio®** are highly efficient in IVIVE. We offer the highest possible stock selection, covering the range of applicable metabolic parameters.

	CD-1 Mouse	**SD Rat**	**Beagle Dog**	**Cynomolgus Monkey**
Liver Homogenate	20	20	20	20
	0,5	0,5	0,5	0,5
Pooled Liver S9 fraction	Negative	Negative	Negative	Negative
	Negative	Negative	Negative	Negative
Pooled Liver Microsomes	Negative	Negative	Negative	Negative
Pooled Suspension Hepatocytes	confirmed	confirmed	confirmed	confirmed

Medium- and Low-Clearance Compounds

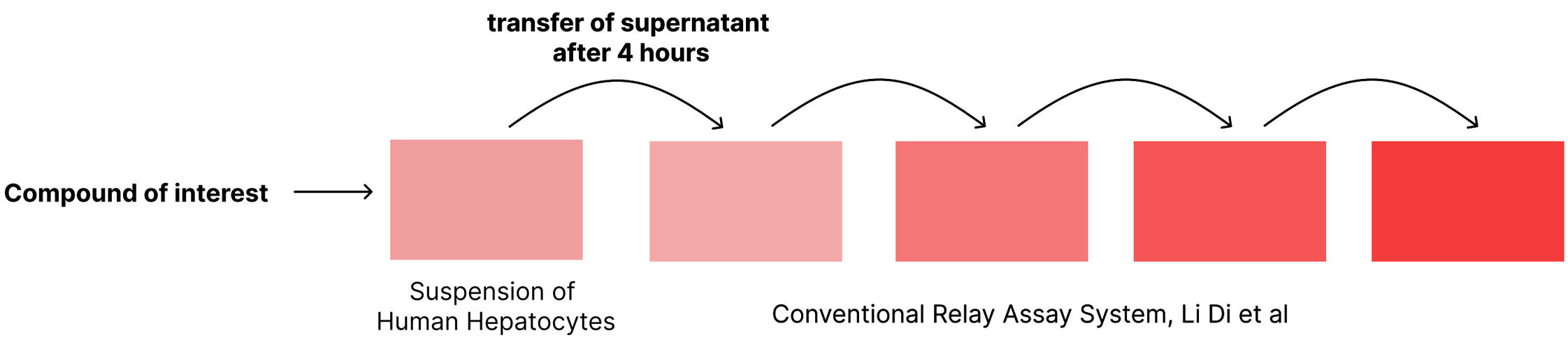
In modern drug discovery, a critical factor in early-stage compound evaluation is how efficiently a candidate drug is metabolized in the liver as well as in other organs. Hepatic clearance plays a central role in determining a drug's pharmacokinetics, safety, and efficacy.

Based on their rate of hepatic elimination, xenobiotics are commonly classified into two categories: high-clearance and low-clearance compounds (LCCs). High-clearance compounds are metabolized rapidly, often at rates approaching hepatic blood flow. Understanding the metabolic fate of these compounds is crucial because LCCs often exhibit prolonged half-lives, leading to drug accumulation, delayed toxicity, or unforeseen drug–drug interactions in clinical settings. In such cases, metabolite identification becomes particularly important, as low-turnover compounds tend to form metabolites slowly and sometimes only after extended exposure. These metabolites—especially when reactive, lipophilic, or conjugated—may be pharmacologically active or toxic, contributing to idiosyncratic adverse effects that are not detectable in early high-clearance-focused assays.

A major challenge with LCCs is their slow interaction with hepatic enzymes, making them difficult to evaluate using conventional in vitro assays such as hepatocyte suspensions or liver microsomes. Despite this, reliable characterization of a compound's metabolic route and outcome is essential during drug development. Early clearance assays can generally distinguish between high- and low-clearance compounds, but more advanced approaches are required for detailed metabolic identification.

To address these limitations, several advanced in vitro systems have been developed. These platforms are generally grouped into the following categories: relay assays, plateable hepatocyte-based models, spheroid-based systems, and microphysiological systems (MPS).

Relay assays involve the repeated incubation of test compounds with fresh suspensions of human or animal hepatocytes over multiple time points (1). Because hepatocytes in suspension remain viable for only a few hours, this approach extends metabolic incubation by transferring the compound to new cell preparations several times, usually 4 or 5. While effective in capturing slowly formed metabolites, the method requires multiple steps and careful coordination.



Plateable hepatocytes can be used either as monocultures or co-cultured with feeder cells to support hepatic functionality (2, 3, 4). These cells can be cryopreserved and pooled, offering improved reproducibility and operational convenience. Their longer-term stability in culture provides valuable insight into compound clearance over extended periods. However, traditional plating methods were proven to lead to decreased cytochrome P450 (CYP) enzyme levels, prompting the development of co-culture techniques aimed at maintaining enzyme activity (2). While much progress has been made, many commercial co-culture systems—particularly those based on feeder layers—require multi-day establishment periods or rely on freshly plated (non-cryopreserved) hepatocytes. As a result, these platforms are difficult to integrate into routine workflows, demanding additional setup time, logistics coordination, and specialized handling. This significantly limits their scalability and practicality for early-stage screening or high-throughput applications, where consistency and operational efficiency are essential.

[1] L. Di and R. S. Obach, "Addressing the Challenges of Low Clearance in Drug Research," AAPS J, vol. 17, no. 2, pp. 352–357, Mar. 2015, doi: 10.1208/s12248-014-9691-7.
[2] I. Hultman, C. Vedin, A. Abrahamsson, S. Winiwarter, and M. Darnell, "Use of HμREL Human Coculture System for Prediction of Intrinsic Clearance and Metabolite Formation for Slowly Metabolized Compounds," Mol Pharm, vol. 13, no. 8, pp. 2796–2807, Aug. 2016, doi: 10.1021/acs.molpharmaceut.6b00396.
[3] D. A. Kukla et al., "Clearance prediction with three novel plated human hepatocyte models compared to conventional suspension assays: Assessment with 50 compounds and multiple donors," Drug Metabolism and Disposition, vol. 53, no. 2, p. 100032, Feb. 2025, doi: 10.1016/j.dmd.2024.100032.
[4] D. F. McGinnity, M. G. Soars, R. A. Urbanowicz, and R. J. Riley, "Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for predicting metabolic clearance.," Drug Metabolism and Disposition, vol. 32, no. 11, pp. 1247–1253, Nov. 2004, doi: 10.1124/dmd.104.000026.

Spheroid cultures represent a 3D model that helps retain hepatocyte function, including CYP activity, over time (5, 6, 7). These models are typically formed from spheroid-qualified hepatocytes that can be cryopreserved. Upon culture, the cells often maintain enzyme activity levels similar to those in suspension cultures, making them useful for studying LCCs. However, the relatively small number of cells in each spheroid may limit the scale and throughput of standard metabolic assays.

MPS platforms, often referred to as "organ-on-a-chip" systems, replicate organ-level complexity by incorporating perfusion and tissue-mimicking structures (8). These models simulate the dynamic environment of the liver, enabling the study of metabolism and pharmacokinetics in a more physiologically relevant context. MPS systems have demonstrated utility in LCC metabolism studies but may require more specialized equipment and expertise, and are generally better suited for low- to medium-throughput applications.

In Vitro System	Advantages	Limitations
Relay Hepatocyte Assays	Captures slow metabolite formation; extends incubation time without losing enzyme activity	Labor-intensive; requires precise timing and multiple cell preparations
Plateable Hepatocytes	Cryopreserved; reproducible; supports longer-term metabolism studies; co-culture options preserve CYP activity	Enzyme activity may decline in monocultures; co-cultures can be complex; variability in cryopreservation
Spheroid Hepatocytes	3D structure enhances cell functionality; retains CYP activity over time; physiologically relevant	Limited cell number per spheroid; lower throughput for quantitative assays
Microphysiological Systems (MPS)	Highly physiologically accurate; supports dynamic flow; ideal for long-term metabolism and PK studies	Requires specialized equipment and expertise; low to medium throughput; costly setup

[5] T. Hurrell et al., "Human Liver Spheroids as a Model to Study Aetiology and Treatment of Hepatic Fibrosis," Cells, vol. 9, no. 4, p. 964, Apr. 2020, doi: 10.3390/cells9040964.
[6] K. P. Kanebratt et al., "Primary Human Hepatocyte Spheroid Model as a 3D In Vitro Platform for Metabolism Studies," JPharmSci, vol. 110, no. 1, pp. 422–431, Jan. 2021, doi: 10.1016/j.xphs.2020.10.043.
[7] L. C. Preiss, V. M. Lauschke, K. Georgi, and C. Petersson, "Multi-Well Array Culture of Primary Human Hepatocyte Spheroids for Clearance Extrapolation of Slowly Metabolized Compounds," AAPS J, vol. 24, no. 2, p. 41, Mar. 2022, doi: 10.1208/s12248-022-00689-y.
[8] S. Ohri, P. Parekh, L. Nichols, S. A. P. Rajan, and M. Cirit, "Utilization of a human Liver Tissue Chip for drug-metabolizing enzyme induction studies of perpetrator and victim drugs," Drug Metabolism and Disposition, vol. 53, no. 1, p. 100004, Jan. 2025, doi: 10.1124/dmd.124.001497.

Preci's in vitro clearance EVH (extended viability hepatocytes) system delivers the metabolic performance of a relay assay within a simplified suspension format. Utilizing extended-clearance human hepatocytes, our platform retains CYP enzyme activity and supports phase I metabolism for up to 12 hours in a single incubation—eliminating the need for repeated cell transfers. This enables robust evaluation of low-clearance compounds with minimal workflow complexity. In addition, the system is based on cryopreserved hepatocytes, allowing convenient storage and batch-to-batch consistency for long-term studies and scalable screening programs.

Preci's extended-clearance hepatocyte (EVH) system is designed to reliably assess both high- and low-clearance compounds within a single, streamlined suspension assay. The high initial enzymatic activity of our pre-qualified and gradient-purified hepatocytes enables rapid metabolism of high-clearance compounds, while the sustained CYP function—retained for up to 12 hours—allows accurate profiling of slow-turnover, low-clearance compounds. This dual capability eliminates the need to switch between assay platforms during early-stage drug development, offering a unified solution for comprehensive hepatic clearance evaluation.

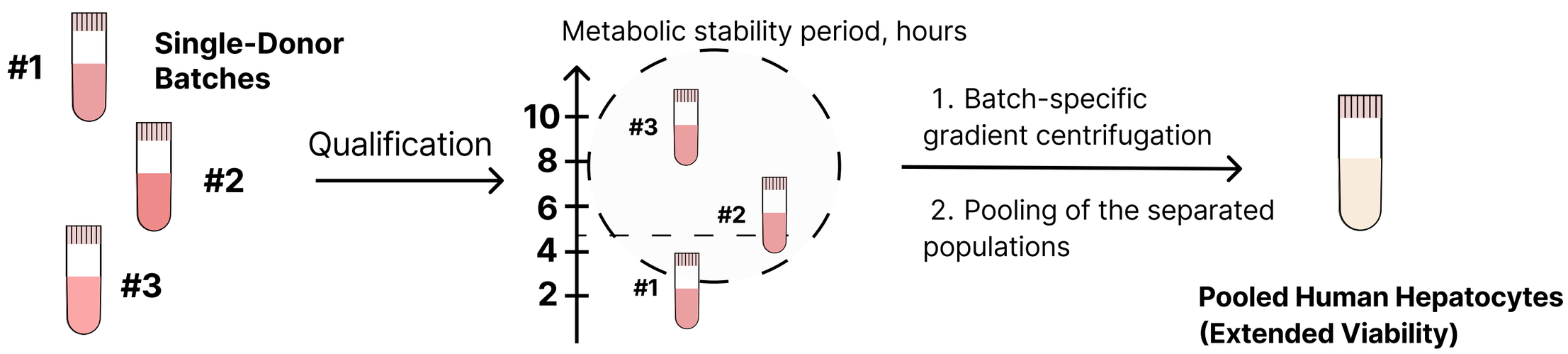
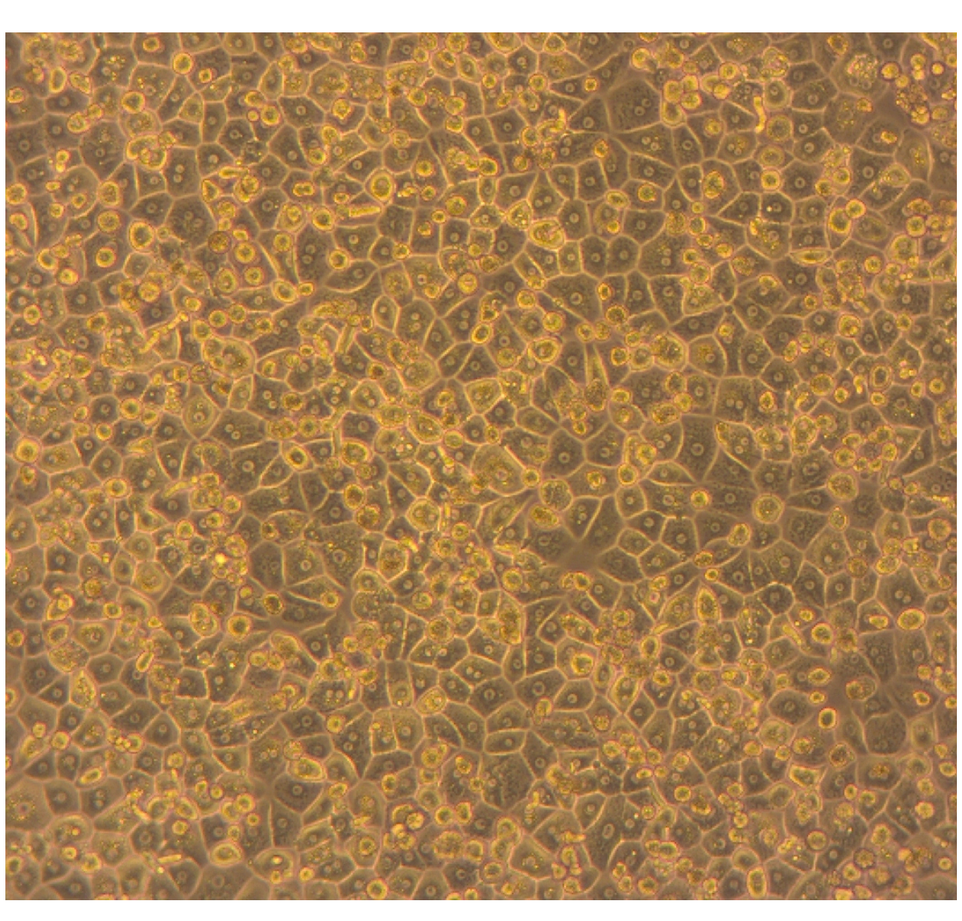


Figure 3. Schematic representation of EVH product pooling procedure

Pooled-5 Plateable Human Hepatocytes (HEP-P-5)



Our **Pooled Plateable Human Hepatocytes** are qualified for metabolic stability assays for at least 72 hours. Those cells are known to contain less active Phase I enzymes then pooled suspension product, however, are good for long-term evaluation of compound clearance

Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	8	11
Viability upon thawing*	85%	100%
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Plateability (confluency)	85%	100%
Monolayer stability	72 hours	
Metabolic parameters		
Phenacetin clearance uL/min/mln cells	3	10

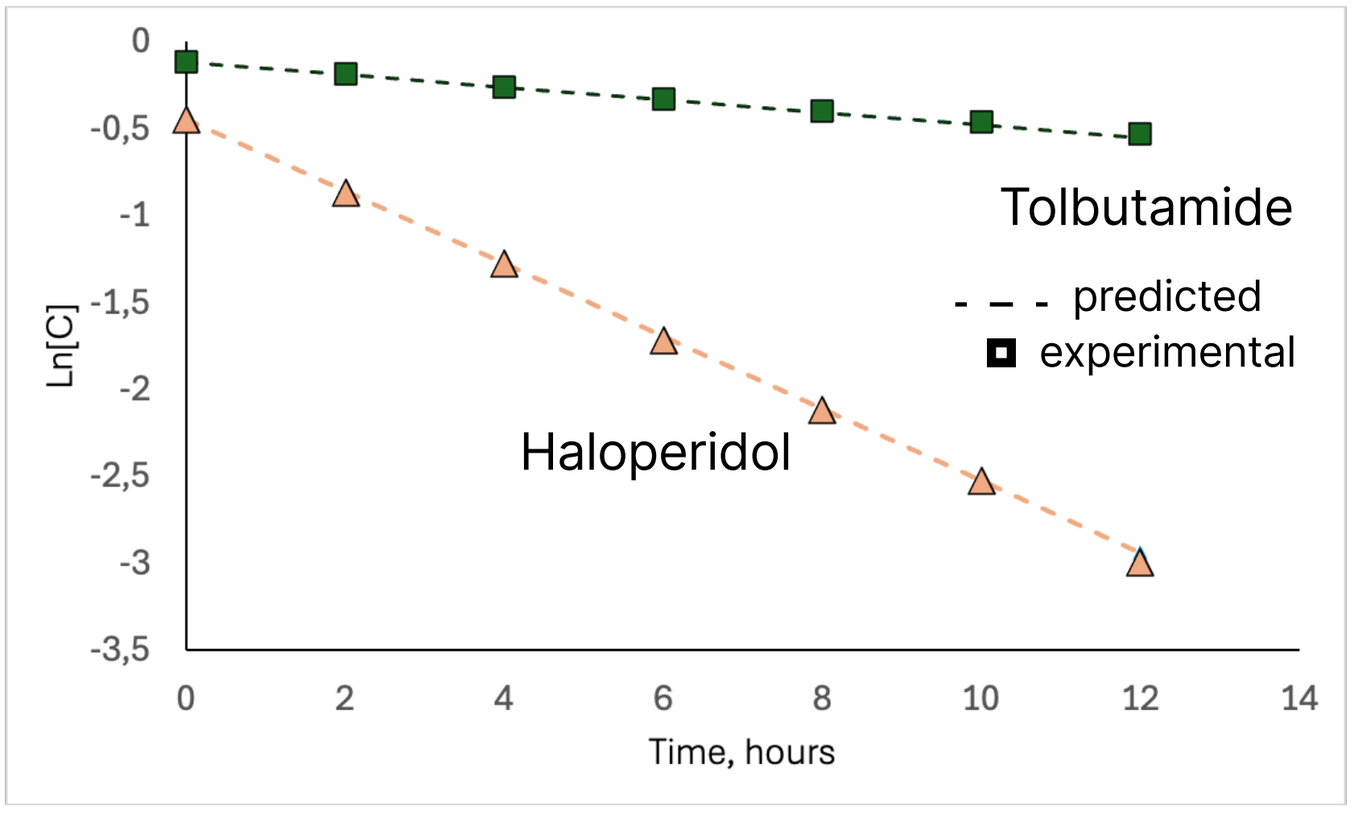
Pooled (pool-5) and Single Spheroid Human Hepatocytes (HEP-P-5)

Our **Pooled Spheroid Human Hepatocytes** and **Single-Donor Spheroid Human Hepatocytes** can be used for both slowly metabolised compounds metabolism and hepatotoxicity. Those cells assemble into either one or several spheroids within 5 days when added to the ultra-low attachment well-plates and later is viable for at least 10 days.

Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	8	11
Viability upon thawing*	85%	100%
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Spheroid formation time	5 days	7 days
Spheroid stability	5 days	12 days
Metabolic parameters		
Phenacetin clearance uL/min/mln cells	3	10

Extended Viability Pooled Suspension Hepatocytes (pool-10) (HEP-S-10)

Our **Extended Viability Pooled Suspension Hepatocytes (EVH)** are pooled per special protocol and can maintain the stable metabolic rate until 12 hours in culture at 0,5 mln cells per incubation. This assay set up allows to avoid the laborious relay assays, with the single incubation just as you use routine Pooled Suspension Hepatocytes products.



Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	6	8
Viability upon thawing	85%	100%
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Metabolic parameters		
Haloperidol, Clint, uL/min/mln (average over 8 hours)	4	8
Tolbutamide, Clint, uL/min/mln (average over 12 hours)	1	2

2.2. Extrahepatic metabolism

While liver remains the main small molecule drug-metabolizing site in the human body, it is not possible to construct the comprehensive PBPK model, especially for new chemical entities, without accounting for gut and kidney. While earlier developed molecules' bioavailability was majorly predicted by Caco-2 or MDCK permeability assay, nowadays it is essential to account for the intestinal metabolism as well as permeability. Intestine is not only highly hydrolytically active tissue reservoir, it also has high levels of CYP enzymes.

Same argument stands for kidney, which also can adsorb and metabolize certain compounds in significant rate, especially for the highly lipophilic compounds. For the hydrolytically unstable compounds, hydrolysis in each tissue reservoir is an essential metabolic rout which needs to be addressed. Mono- and diamine drugs is an important exemption, since they can be metabolized in the brain, which is frequently the site of actions of those molecules.

Metabolism in plasma is highly important for esters, amides, including peptide drugs, and some of the peptidomimetics. Plasma contains metalloproteases, serine proteases, as well as esterases that can promptly engage in the compound cleavage.

For aerosol methods of delivery of drugs or drugs which act in lung tissue, the lung metabolism is also essential. Despite the rate of this process is significantly slower than in liver and kidney, it is sometimes essential to account for in specific cases. In case of skin-permeable molecules keratinocytes can act as drug-metabolizing cells, as well important to estimate the real compound bioavailability for both drugs and cosmetics.

Phase II metabolism in tissue reservoirs is a frequent phenomenon for the covalent inhibitors, which can easily be conjugated with glutathione, and deactivated. Therefore, for those drugs S9 and cellular stability testing is essential.

Integration of these data into PBPK frameworks requires determination of intrinsic clearance (CLint), enzyme kinetic parameters (K_m, V_{max}), and permeability coefficients across the relevant tissues. Comparative scaling of microsomal or S9 fraction activity enables prediction of tissue-specific extraction ratios.

To experimentally dissect these extrahepatic contributions, tissue-derived subcellular fractions and primary cells can be employed—such as intestinal microsomes or enterocytes for gut metabolism, renal proximal tubule cells or kidney microsomes for renal metabolism, and keratinocyte-based systems for dermal metabolism. Preci's portfolio of human-derived fractions allows direct comparison of hepatic versus extrahepatic metabolism within harmonized assay conditions.

Microsomes

Organ	Packaging	Specifications
Lung – non-smoker	10 mg/mL, 0,5 mL/vial	Phenacetin, Clint < 3 uL/mg/min
Lung – smoker	10 mg/mL, 0,5 mL/vial	Phenacetin, Clint 3-5 uL/mg/min
Intestine (with PMSF)	10 mg/mL, 0,15 mL/vial	NADPH CCR>20 U/mg, Verapamil, Clint>20 uL/min/mg
Intestine (without PMSF)	10 mg/mL, 0,15 mL/vial	NADPH CCR >20 U/mg,
Kidney	10 mg/mL, 0,5 mL/vial	NADPH CCR >50 U/mg, Verapamil, Clint>20 uL/min/mg

S9 fractions

Organ	Packaging	Specifications
Lung – non-smoker	5 mg/mL, 1 mL/vial	Phenacetin, Clint < 1 uL/mg/min
Lung – smoker	5 mg/mL, 1 mL/vial	Phenacetin, Clint 1-3 uL/mg/min
Intestine (with PMSF)	4 mg/mL, 1 mL/vial	NADPH CCR>20 U/mg, Verapamil, Clint>20 uL/min/mg
Intestine (without PMSF)	4 mg/mL, 1 mL/vial	NADPH CCR >20 U/mg,
Kidney	5 mg/mL, 1 mL/vial	NADPH CCR >20 U/mg, CYP3A4 activity - confirmed
Skin	4 mg/mL, 1 mL/vial	CES1 activity - present

Homogenate

Organ	Packaging
Lung – non-smoker	1:3, 1 mL/vial
Lung – smoker	1:3, 1 mL/vial
Intestine (with PMSF)	1:3, 1 mL/vial
Intestine (without PMSF)	1:3, 1 mL/vial
Kidney	1:3, 1 mL/vial
Skin	1:3, 1 mL/vial
Cornea	1:3, 1 mL/vial
Brain	1:3, 1 mL/vial

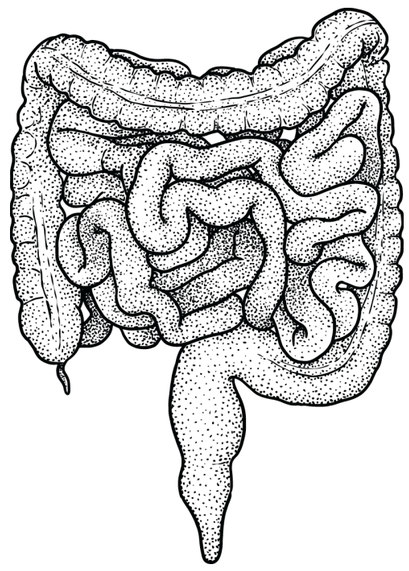
Tissue-derived microsomes, S9 fractions, and homogenates are the essential tools to study compound Phase I and partly Phase II metabolism outside of the liver. Unlike liver microsomes, those organs contain different distribution of drug-metabolizing enzymes. We qualify tissue-derived microsomes based on the activity levels in typical metabolic setting. However, we gladly accept customer-specific testing sets to validate the applicability of specific product to the validation set.

Pooled Suspension Human Keratynocytes (Pool-10) (KER)

Human Keratinocytes are the major actors in xenobiotics metabolism in skin tissues. We offer the unique pooled suspension keratinocytes products for skin drug metabolism assessment and metabolic profiling for dermally administered compounds.

Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
Clopidogrel metabolism	confirmed	confirmed

Pooled Suspension Human Enterocytes (Pool-10) (ENT)



Human Enterocytes are the major actors in gut compound metabolism, and we carefully isolate and package those as Pooled Suspension Human Enterocytes (Pool-10) that can be used just like analogues, and, probably, well-familiar hepatocytes models. We validate CYP3A activity in those cells as well as CES2 activity to match the metabolic properties to intestinal cells.

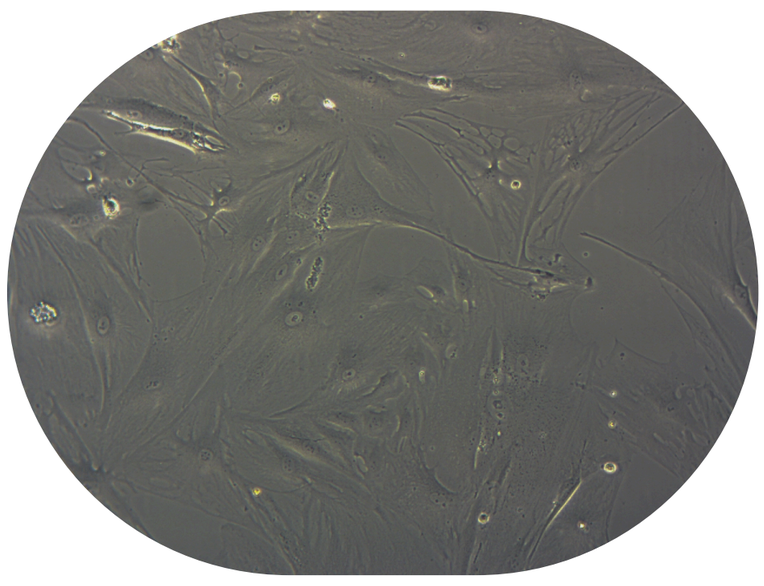
Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
HBV, via qPCR	Negative	
HCV, via qPCR	Negative	
HIV, via qPCR	Negative	
Metabolic parameters		
Verapamil, Clint, uL/min/mln (average over 8 hours)	15	30
Irinotecan metabolism	confirmed	confirmed

he concept of F_{gut} represents an important refinement in pharmacokinetic modeling, serving as an alternative or complement to the classical bioavailability term (F), which reflects the fraction of the administered dose reaching systemic circulation. While F encompasses all presystemic losses, including gut and hepatic metabolism as well as incomplete absorption, Fgut isolates the specific contribution of intestinal metabolism to first-pass extraction. This parameter becomes crucial for compounds extensively metabolized by enteric CYP enzymes, esterases, or amidases before reaching the portal vein. Experimentally, gut metabolism can be evaluated analogously to intrinsic clearance (Clint) measurements in hepatocytes, using uspension cultures of human enterocytes. By quantifying the depletion rate or metabolite formation kinetics, the intestinal intrinsic clearance ($C_{lint,gut}$) can be estimated and incorporated into physiologically based pharmacokinetic (PBPK) models to refine the prediction of oral bioavailability and systemic exposure.

2.3. Compound permeability and deposition models

Despite Caco-2 and MDCK cell models remain perfectly suited solution for most of bioavailability measurements at the early stages of drug development, they do not account for some of the transporter effects and metabolism that occurs in the natural barriers.
Therefore, for more demanding applications, we offer the range of the primary cell human-derived products, which ought to bring additional insights into small molecule drug permeability and deposition. Despite no models can accurately re-create the real barrier structure accounting for the complex tissue nature, some models can approach the level of complexity.
We pay the special attention conserve both metabolic activity and permeability of the formed cellular monolayes, which simulate the real microstructure of the barrier. Plateable human enterocytes, extracted from intestinal explants proliferate in plateable format and form the cellular monolayer with several weeks. Those cells can be used in analogues ways to Caco-2 model, however, in less high throughput manner and for closer modeling of intestinal wall permeation.
Skin models are highly studied, and skin disks remain the major tool for the drug adsorbtion through skin are skin disks layered on the Lorenz chamber.

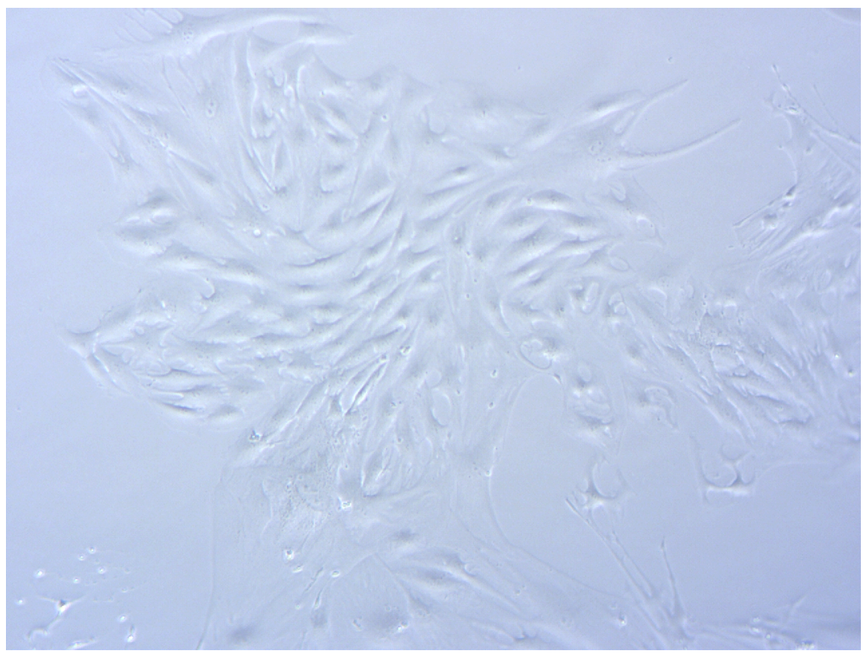
Plateable Human Enterocytes (ENT)



Plateable human enterocytes exhibit active metabolic and transport functions, including CYP450 enzyme activity and the presence of efflux and uptake transporters, making them ideal for studying intestinal absorption, first-pass metabolism, and drug–nutrient interactions. Upon plating, they form tight junctions and display stable morphology over several days in culture, providing a reliable platform for drug permeability, metabolism, and safety assessment studies, as well as physiologically relevant PBPK model input generation.

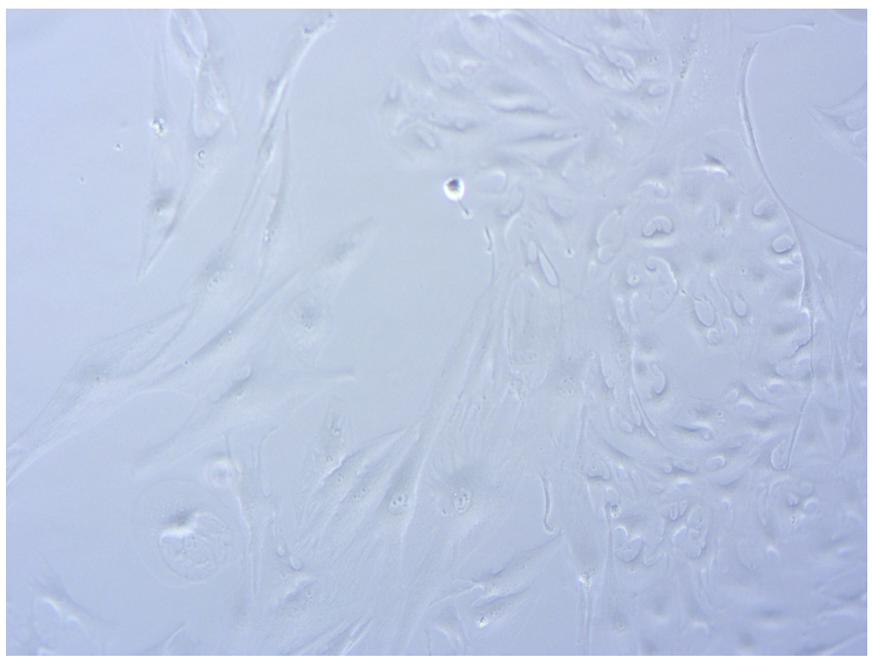
Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
Monolayer formation	confirmed	confirmed

Plateable Human Keratynocytes (KER)



Keratinocytes are the primary cell type forming the human epidermis and play a central role in maintaining skin barrier function, wound healing, and xenobiotic metabolism. They represent an essential in vitro model for studying dermal absorption, irritation, and toxicity. Our **plateable human keratinocytes** readily attach and proliferate in culture, forming uniform stratified monolayers suitable for drug permeability, irritation response, and metabolic activity assessment. These cells provide a physiologically relevant platform for topical formulation testing and dermal exposure research.

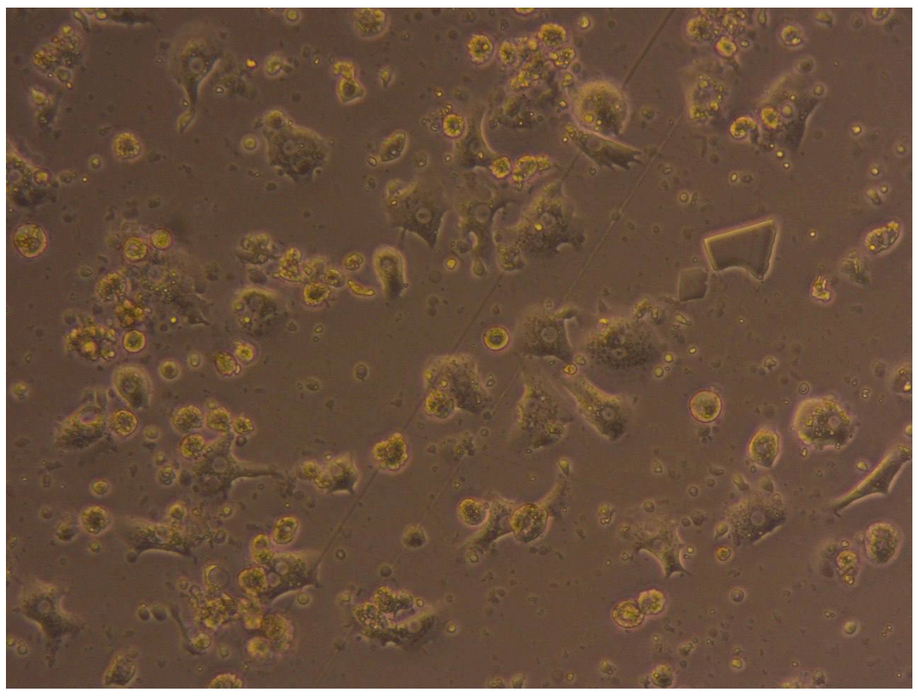
Dermal Fibroblasts (FIB)



Human dermal fibroblasts are the essential stromal component supporting the skin barrier. In vitro, plateable dermal fibroblasts serve as a crucial complement to keratinocyte-based skin models, ensuring proper epidermal differentiation and response to external stressors. Their metabolic and signaling activity plays a vital role in modeling dermal reactions to topically applied compounds, irritation, and fibrosis, making them indispensable for assessing dermal toxicity, wound healing potential, and long-term skin safety of cosmetic and pharmaceutical formulations.

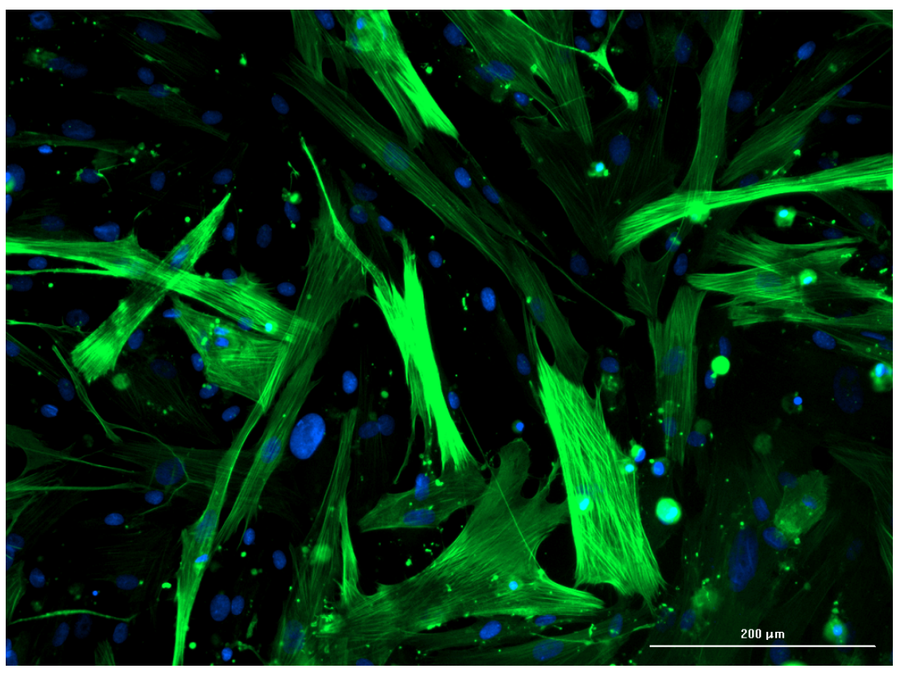
Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
Monolayer formation	confirmed	confirmed

Primary Human Kupffer Cells (KER)



Kupffer cells are essential in ADME studies of large molecules, as they actively mediate hepatic uptake, clearance, and degradation of biologics, nanoparticles, and antibody–drug conjugates through phagocytic and endocytic mechanisms, critically influencing their hepatic deposition and systemic distribution.

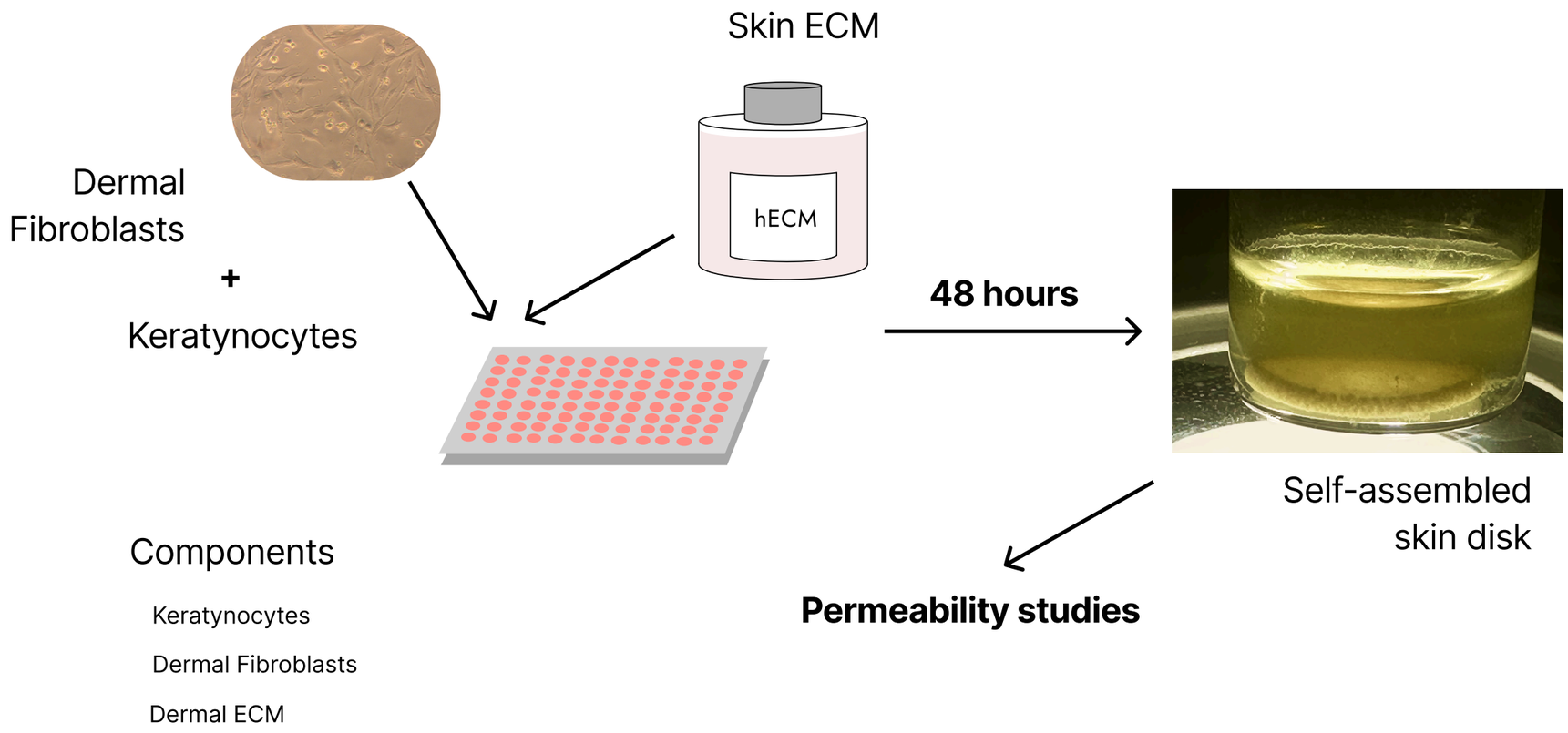
Primary Human Stellate Cells (STE)



Hepatic stellate cells are key regulators of extracellular matrix turnover and vitamin A storage in the liver, playing a central role in maintaining hepatic architecture. In ADME studies, they are important for evaluating large molecule and nanoparticle deposition, as well as fibrosis-related changes that can alter drug distribution and clearance.

Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
Monolayer formation	confirmed	confirmed

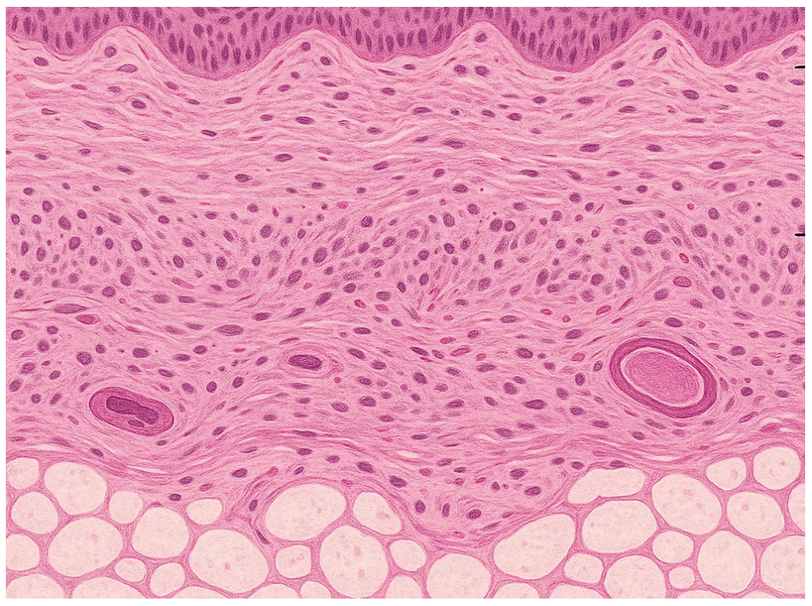
Cryoskin model



Cryoskin represents a solution to the long-standing challenge of having physiologically relevant skin models that can be cryopreserved and conveniently used on demand for permeability and irritation studies. Conventional skin equivalents often suffer from limited shelf-life and poor reproducibility after thawing, which complicates their use in routine toxicology or formulation workflows. By addressing the unmet need for standardized, ready-to-use human-derived skin models, **Cryoskin** ensures experimental consistency and enables efficient integration into screening pipelines for dermal exposure, irritation, and absorption testing.

This innovative product is derived through a self-assembly process involving dermal fibroblasts, keratinocytes, and the extracellular matrix from the same donor, ensuring donor-specific molecular integrity and barrier fidelity. Upon reconstitution, **Cryoskin** rapidly regains the stratified architecture and barrier characteristics of native human skin, making it suitable for early-stage permeability and skin toxicity evaluations. It serves as a reliable platform to estimate compound absorption, irritation potential, and formulation effects before proceeding to more complex or costly in vivo studies.

Skin discs



Disc Size:

Standard: Ø 3.5 cm (custom sizes available)

Thickness: 750 µm (custom sizes available)

Suited for

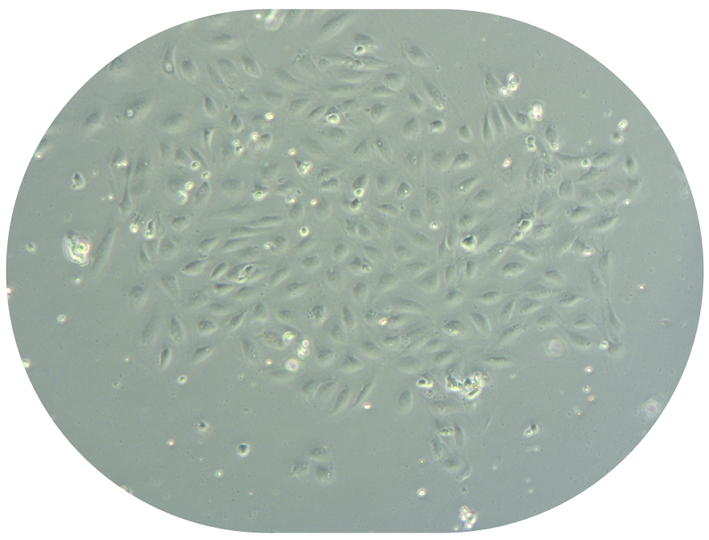
Percutaneous absorption and permeation studies

Topical drug delivery and formulation testing

Regulatory toxicology (OECD guideline support)

Frozen Skin Discs are precision-cut, cryopreserved tissue samples designed for skin permeability assays, offering an ideal solution for researchers in the fields of pharmacology, toxicology, and dermatology. These discs serve as a reliable ex vivo model for evaluating transdermal drug absorption and dermal delivery, delivering consistent and high-quality results in your research. Ideal for In Vitro Permeation Testing (IVPT): Optimized for use in Franz diffusion cells, our discs provide an excellent model for in vitro skin absorption testing. Compliant with international standards, including international standards, they are trusted for regulatory studies and drug formulation development.

Human Umbevical Cord Epithelial cells (HUVEC)

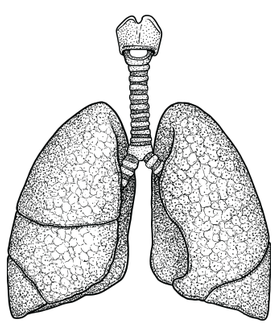


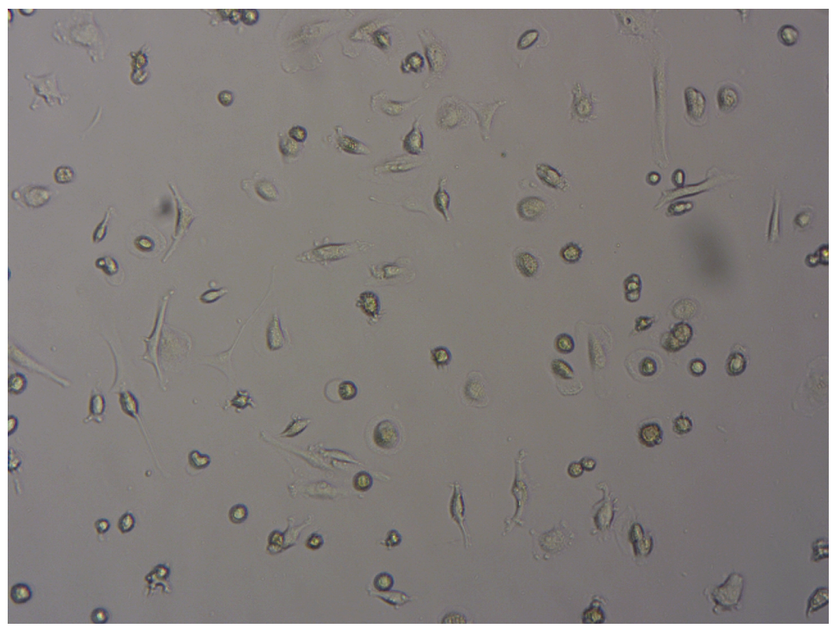
Human umbilical vein endothelial cells (HUVEC) are primary endothelial cells derived from the interior lining of the umbilical vein. HUVECs provide a reliable system to investigate mechanisms of vascular permeability, inflammation, thrombosis, and drug-induced vascular toxicity. Due to their robust growth and physiological relevance, they serve as a standard in vitro model for evaluating compounds affecting endothelial barrier integrity, nitric oxide signaling, and cell–cell adhesion pathways.

Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
Passage number	P1	P2

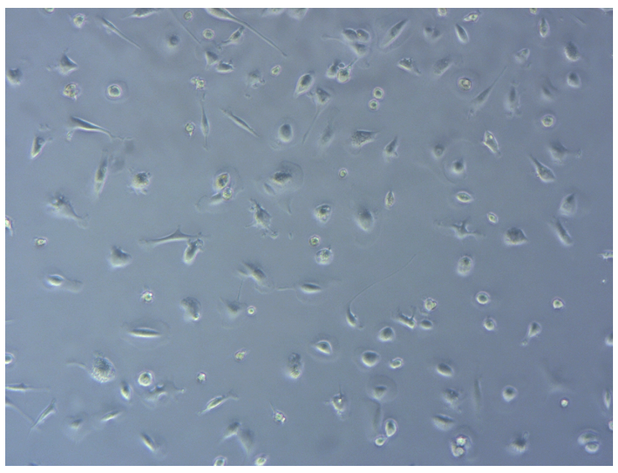
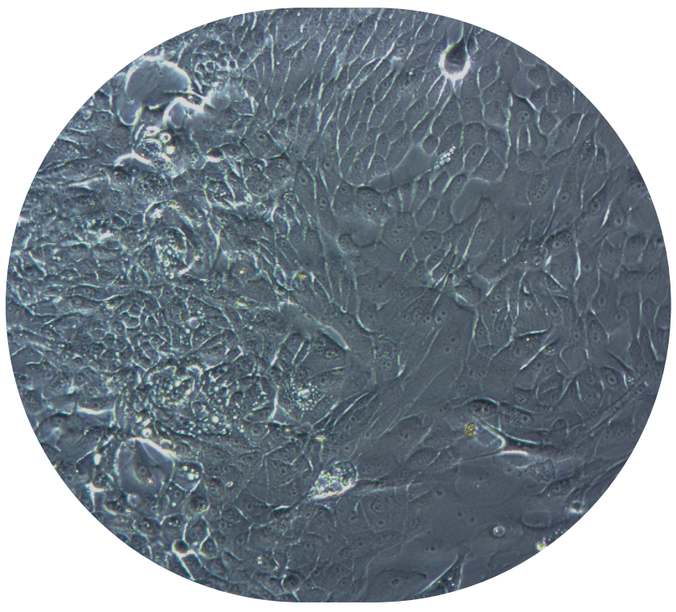
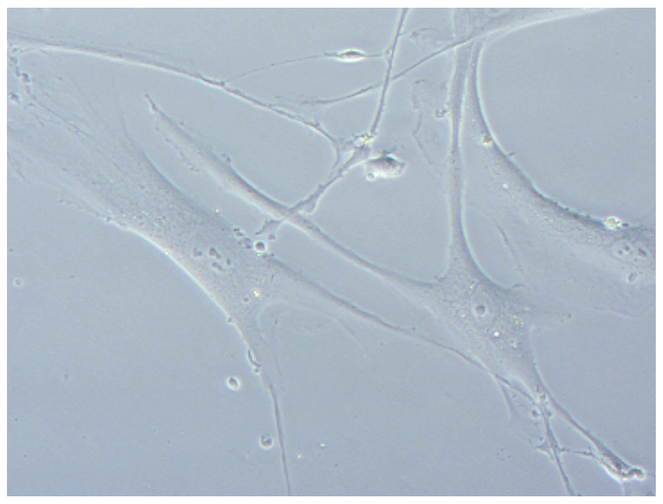
Alveolar Epithelial Cells (AEC)

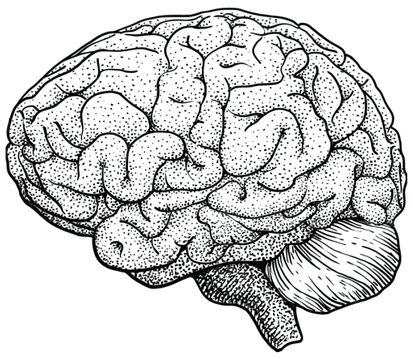
Alveolar epithelial cells (AEC) are primary cells derived from the distal lung region, responsible for gas exchange and barrier maintenance. In vitro, AECs can differentiate at the air–liquid interface (ALI) to form polarized monolayers that closely resemble the native alveolar barrier. These cultures develop tight junctions and distinct Type I/Type II phenotypes, making them ideal for modeling pulmonary permeability, drug absorption, and aerosolized compound delivery. AEC-based ALI models are widely applied in respiratory drug screening, nanoparticle transport studies, and evaluation of inhaled formulation safety and efficacy.

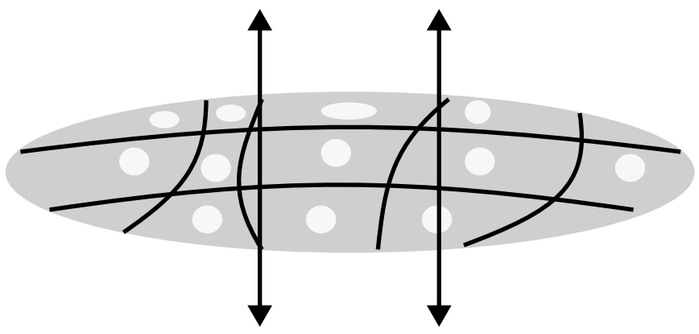




Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	1	1,5
Viability upon thawing	85%	100%
Metabolic parameters		
ALI interface monolayer formation	P2	P3

Bronchial epithelial cells	Small airway epithelial cells	Lung fibroblasts
		

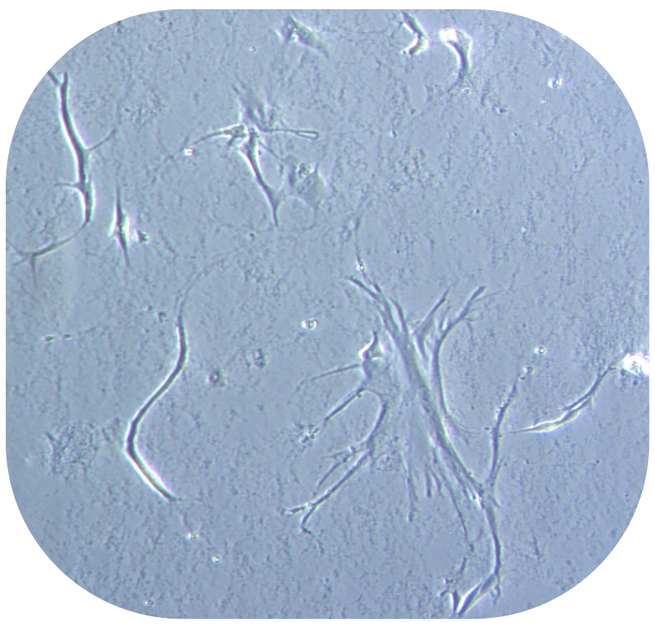




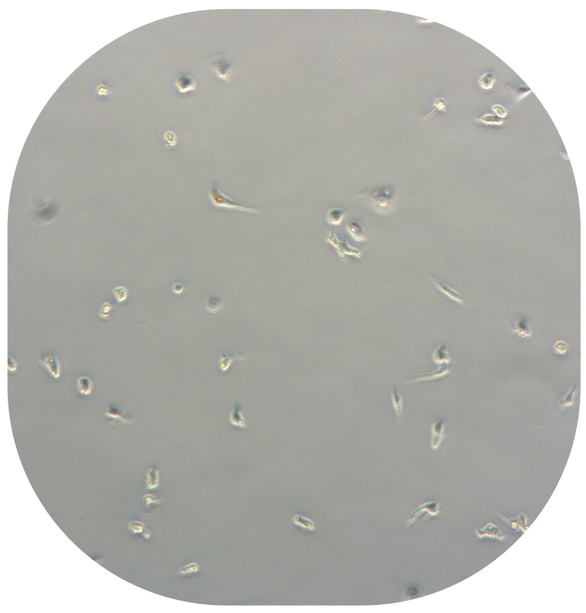
BBB permeability studies

Brain metabolism studies

Human Astrocytes



Human Brain Microvascular Endothelial cells



Blood–brain barrier (BBB) permeability studies are a critical component of ADME profiling for molecular candidates intended to reach or avoid the central nervous system. The BBB serves as a highly selective interface that regulates molecular transport between the circulation and the brain, governed by tight junctions, active efflux transporters such as P-gp and BCRP, and metabolic enzymes. Evaluating compound permeability across the BBB, often using in vitro models such as primary brain endothelial monolayers or co-culture systems with astrocytes, allows prediction of CNS exposure and drug distribution.

Beyond transport, the brain possesses distinct metabolic capacity, involving oxidative enzymes, amidases, and monoamine oxidases that can alter drug stability and bioavailability locally. Therefore, integrating BBB permeability data with brain microsomal stability and cellular uptake assays is essential to understand both pharmacokinetic behavior and potential neurotoxicity of new molecular entities.

2.5. Plasma and Blood clearance, bioanalytical matrices

Plasma protein binding (PPB) is the crucial assay during the development of new molecules for biomedical use. Preci provides high-quality bioanalytical matrices, which can serve as tools to determine protein binding, matrix effects, and compound clearance. Anticoagulants can significantly impact the plasma properties, therefore we offer the wide portfolio of plasma products, starting with **K2EDTA-supplemented plasma for PPB**, and **NaHeparinate and LiHeparinate plasma for clearance** experiments. Various volume-depended discounts may apply to those products. We qualify the products based on the applicability in both PPB and plasma clearance, based on common substrates for this goal.

Ultrafiltered plasma with 30 kDa cut-off can be used for study of non-albumin binding in plasma and interaction of molecules with small proteins present in the matrix.

Products in stock	Product code
Plasma Pharmacokinetic Research System (Human Plasma), K2EDTA, Pooled	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), Li Heparin, Pooled	HPL-LIH
Plasma Pharmacokinetic Research System (Human Plasma), Na Heparin, Pooled	HPL-NAH
Plasma Pharmacokinetic Research System (Human Plasma), K3EDTA, Pooled	HPL-K3
Plasma Pharmacokinetic Research System (Human Plasma), Hemolyzed, Pooled	HPL-HEM
Plasma Pharmacokinetic Research System (Human Plasma), Lipemic, Pooled	HPL-LIP
Plasma Pharmacokinetic Research System (Human Plasma), Sodium Citrate, Pooled	HPL-CIT
Plasma Pharmacokinetic Research System (Human Plasma), Ultrafiltrated 30kDa, Pooled	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), K2EDTA, Individual	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), Li Heparin, Individual	HPL-LIH
Plasma Pharmacokinetic Research System (Human Plasma), Na Heparin, Individual	HPL-NAH
Plasma Pharmacokinetic Research System (Human Plasma), K3EDTA, Individual	HPL-K3

IgG-depleted matrices

For demanding quantification of ADC metabolism and binding in plasma without accounting for anti-IgG antibodies we offer **IgG-depleted and all immunoglobulin-depleted plasma** and serum for such assays. Immunoglobulin depletion occurs through the affinity chromatography ensuring the high efficiency of Ig removal.

Products in stock

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, Platelet-rich, Pooled

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, IgG-depleted, Pooled

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, IgG, IgM, IgE, IgA - depleted, Pooled

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, Platelet-rich, Individual

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, IgG-depleted, Individual

Plasma Pharmacokinetic Research System (Human Plasma), 100 mL, IgG, IgM, IgE, IgA - depleted, Individual

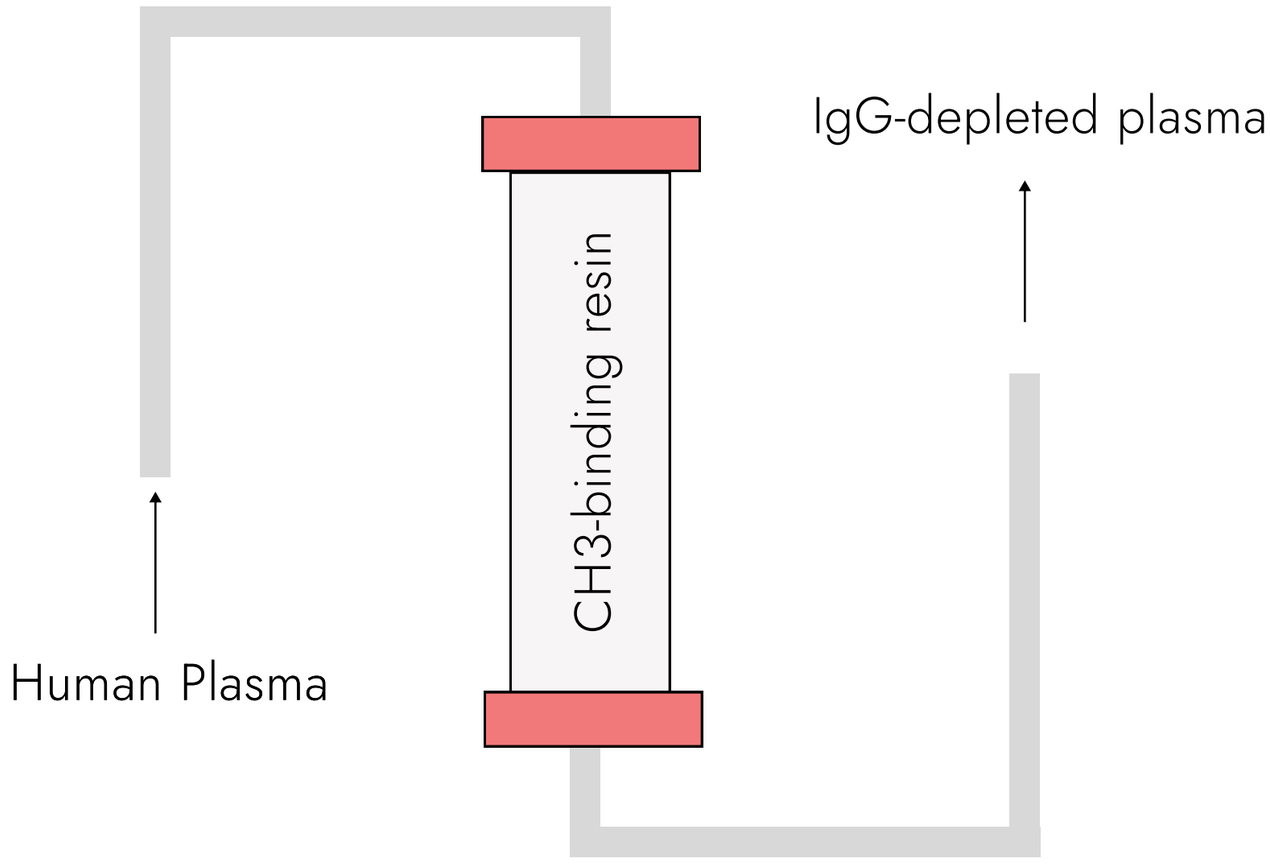
Serum Pharmacokinetic Reasearch System (Human Serum), 100 mL, IgG-depleted, Pooled

Serum Pharmacokinetic Reasearch System (Human Serum), 100 mL, IgG, IgM, IgE, IgA - depleted, Pooled

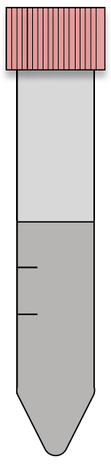
Serum Pharmacokinetic Reasearch System (Human Serum), 100 mL, IgG-depleted, Pooled

Serum Pharmacokinetic Reasearch System (Human Serum), 100 mL, IgG, IgM, IgE, IgA - depleted, Pooled

Complete IgG plasma depletion is achieved through chromatography on a CH3-binding resin, which selectively captures immunoglobulin G molecules via their Fc region, allowing efficient removal of IgG while preserving the native composition and activity of other plasma proteins.



Cerebrospinal fluid (CSF)



If you wish to study the matrix effects on the molecule in **CSF**, we also offer the CSF-derived products from both healthy and diseased donors in large quantities, enabling the high-quality reproducible assay read-outs.

Products in stock

Human Cerebrospinal Fluid, 20 mL, Pooled, Healthy Donor CSF

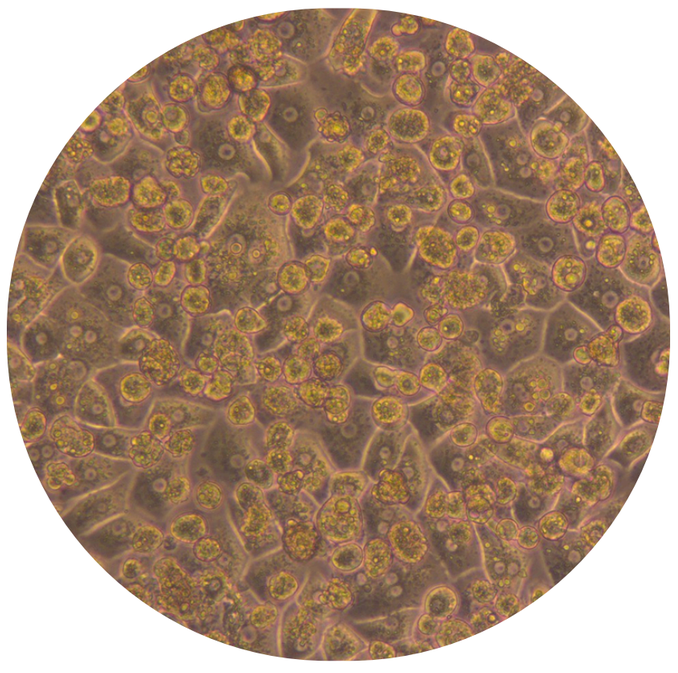
Human Cerebrospinal Fluid, 20 mL, Individual, Healthy Donor CSF

Human Cerebrospinal Fluid, 20 mL, Pooled, Alzeheimer Disease CSF

Human Cerebrospinal Fluid, 20 mL, Individual, Alzheimer Disease CSF

2.5. Drug-drug interactions and transporter studies

Evaluation of CYP induction is the important element in the IND-enabling drug candidate evaluation. ICH M12 prescribes using the **plateable single-donor hepatocytes** pre-qualified using reference inducers and mRNA expression increase read-out for CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19. We provide such hepatocytes in the large batch sizes, to ensure the consistency among the multiple assays.



Induction-Qualified Plateable Human Hepatocytes

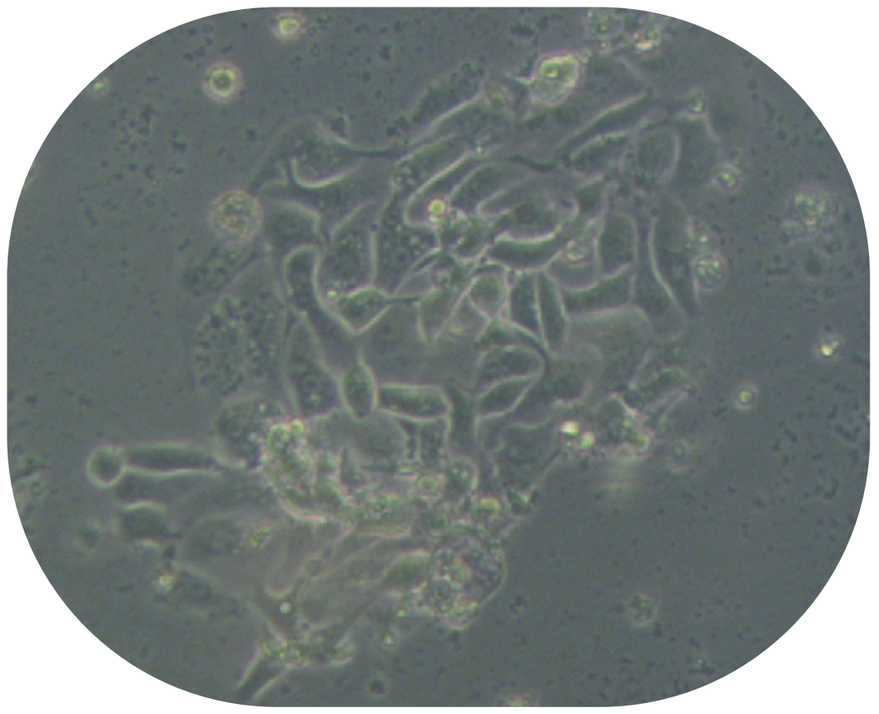
Feature	Specification value, min	Specification value, max
General		
Number of Cells/vial, mln	7	11
Viability upon thawing	85%	100%
CYP1A2 induction with Omeprazole (mRNA level)	10	
CYP2B6 induction with Phenobarbital (mRNA level)	10	
CYP3A4 induction with Rifampicine (mRNA level)	10	
CYP2C8, CYP2C9, CYP2C19 induction with Rifampicine (mRNA level)	2	

Our **Induction-Qualified Plateable Human Hepatocytes** provide a reliable and human-relevant platform specifically optimized for drug–drug interaction (DDI) assessments. Each donor lot is rigorously screened for inducibility across key CYP isoforms—CYP1A2, CYP2B6, CYP3A4, as well as CYP2C8, CYP2C9, and CYP2C19—ensuring consistent and predictable metabolic responses to prototypical inducers and inhibitors. With stable plateability, maintained polarity, and sustained enzyme functionality, these hepatocytes enable accurate evaluation of induction- and inhibition-driven DDIs and support both mechanistic and regulatory-oriented metabolic interaction studies.

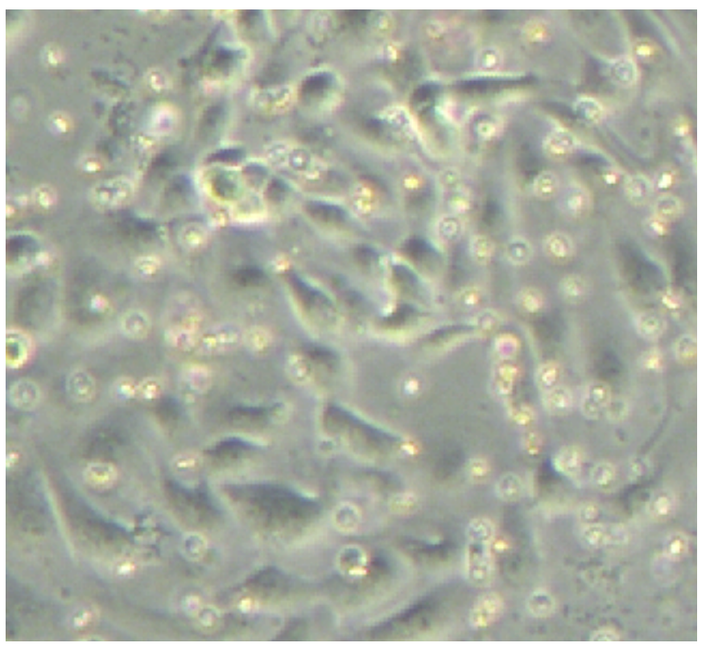
Transport modelling products

Renal proximal tubule epithelial cells (PTEC)

Renal proximal tubule epithelial cells (RPTEC) represent the primary functional cells of the kidney's proximal tubule, responsible for reabsorption, secretion, and metabolism of solutes, drugs, and xenobiotics. They are highly relevant for modeling renal drug transport, nephrotoxicity, and metabolic stability in vitro. RPTECs express key transporters and enzymes such as OATs and CYPs, making them ideal for mechanistic studies of drug clearance, transporter interactions, and renal-specific toxicity screening in both preclinical and translational research settings.



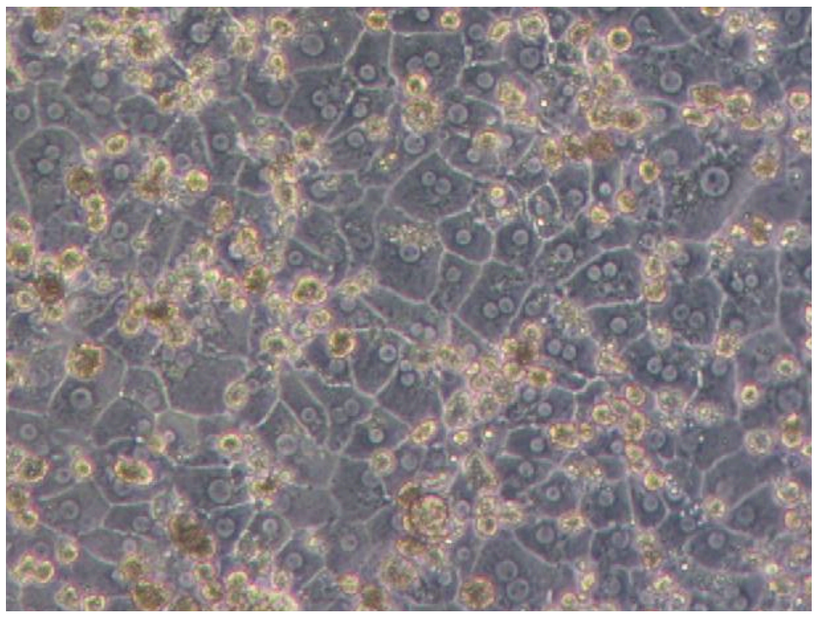
Renal glomerular endothelium cells (GEC)



Renal glomerular endothelial cells (GEC) line the capillaries of the glomerulus and form a crucial part of the kidney's filtration barrier. These cells are essential for studying glomerular permeability, nephrotoxicity, and renal drug transport mechanisms. GECs are frequently applied in in vitro models for investigating kidney-specific vascular function, diabetic nephropathy, and inflammatory or immune-mediated renal injury. They serve as a valuable tool for drug screening, toxicity testing, and mechanistic research on renal microvascular health and disease.

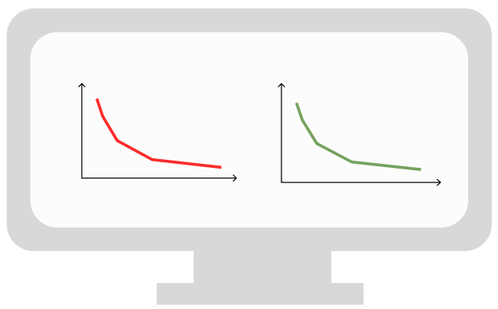
Transporter-Qualified Human Hepatocytes

Transporter-qualified human hepatocytes are a key system in ADME profiling, particularly for understanding drug uptake, biliary excretion, and intracellular accumulation driven by active membrane transport processes. These hepatocytes maintain functional expression of key uptake transporters such as OATP1B1, OATP1B3, and NTCP, as well as efflux transporters like BCRP, MRP2, and P-gp, allowing detailed evaluation of transporter-mediated clearance mechanisms. Studying molecular candidates in these cells helps identify whether hepatic disposition is transporter-dependent and to what extent drug–drug interactions may occur.



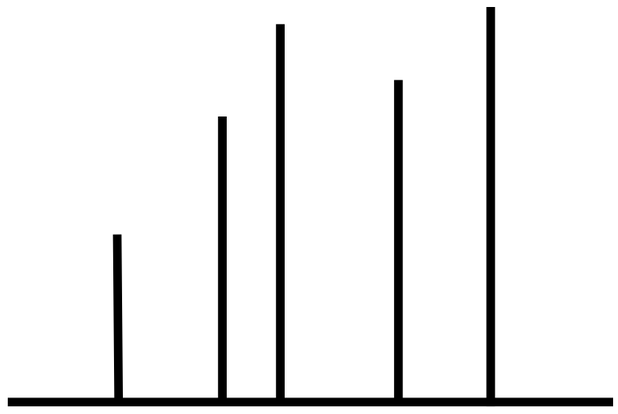
2.6. Custom Services

In case custom substrate-clearance benchmarks are required or you wish to avoid additional batch validation, Preci can screen your full validatory set—up to 50 compounds—against our microsomes or hepatocytes and deliver the complete data package. We can meet your internal validation standards with our pooled products by first testing each single-donor batch against your validatory panel and then designing the final pool composition to match the required clearance profile. If metabolite profiling is part of your validation workflow, Preci can perform this assay on our stocked batches and provide detailed metabolite patterns to guide precise product selection. For biofluids, we can also validate your binders or substrates against our matrices, ensuring consistent performance and eliminating the need for repeated product re-validation.



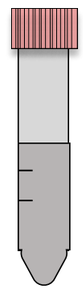
Substrate clearance rate
Metabolite formation rate

up to 50 compounds in hepatocytes/microsomes validation set **included into hepatocytes/ microsomes batch price**



m/z

Metabolite formation pattern, using HRMS



PPB/plasma clearance assays

Matrix binding assays

Permeability assays

3.1. Tissue cross-reactivity

Tissue cross-reactivity is a highly informative experiment in the antibody specificity testing. It can predict the unwanted binding to off-target sites across the body. Routinely, 33-36 various tissues are used for this assay, with Optimal Cutting Temperature Compound (OCT compound) being preferable storage format. Nevertheless, some studies prefer using Formalin-Fixed Paraffin-Embedded (FFPE) format of tissue preservation.

Preci decided to simplify the tissue sourcing for the TCR studies and provide the unified tissue set, designed for TCR studies. Tissues are collected from surgical materials or cadavers with low ischemia time (<6 hours) to maintain the proteomic contents of the material.

Name	Frozen-Tissue (0.5 g), USD	FFPE, USD	OCT-Embedded Tissue, USD	OCT-Embedded Tissue + FFPE, USD
Adrenal gland	930	600	980	1180
Bladder	698	450	748	948
Bone marrow	930	600	980	1180
Breast	698	450	748	948
Cerebellum	930	600	980	1180
Cerebrum	930	600	980	1180
Cervix	698	450	748	948
Colon	698	450	748	948
Diaphragmatic muscle	698	450	748	948
Esophagus	698	450	748	948
Heart	698	450	748	948
Kidney	698	450	748	948

Name	Frozen-Tissue (0.5 g), USD	FFPE, USD	OCT-Embedded Tissue, USD	OCT-Embedded Tissue + FFPE, USD
Larynx	698	450	748	948
Liver	698	450	748	948
Lung	698	450	748	948
Lymph node	930	600	980	1180
Medulla oblongata	930	600	980	1180
Ovary	698	450	748	948
Pancreas	698	450	748	948
Peripheral nerve	930	600	980	1180
Placenta	698	450	748	948
Prostate	698	450	748	948
Salivary gland	930	600	980	1180
Skeletal muscle	698	450	748	948
Skin	698	450	748	948
Small intestine	698	450	748	948
Spleen	930	600	980	1180
Stomach	698	450	748	948
Testis	930	600	980	1180
Thymus	930	600	980	1180

Name	Frozen-Tissue (0.5 g), USD	FFPE, USD	OCT-Embedded Tissue, USD	OCT-Embedded Tissue + FFPE, USD
Thyroid	930	600	980	1180
Tonsil	930	600	980	1180
Uterus	698	450	748	948
1 complete set	22 134	14 280	23 536	29 146
3 complete sets	50 220	32 400	53 420	66 220

3.2. Plasma and Blood clearance

Our LiHep plasma and IgG-depleted plasma sets are fully validated for ADC, oligonucleotide, and monoclonal antibody stability experiments, offering a highly controlled yet biologically relevant environment for biotherapeutic assessment. These matrices are proteolytically active and maintain native peptidase and esterase activity profiles, ensuring that degradation pathways observed in vitro accurately reflect those encountered in human circulation.

Beyond standard stability testing, these plasma sets are suitable for comparing linker stability in ADCs, evaluating nuclease-driven oligonucleotide cleavage, and assessing monoclonal antibody susceptibility to fragmentation or CDR-region proteolysis. Because IgG-depleted plasma minimizes background immunoglobulin interference, it provides clearer analytical windows for PK, immunogenicity-related degradation, and payload release studies.

Together, these products deliver a reliable platform for predictive biotherapeutic metabolism, catabolism, and formulation screening, helping researchers generate reproducible and translatable data during preclinical development.

Products in stock	**Product code**
Plasma Pharmacokinetic Research System (Human Plasma), IgG-depleted, Pooled	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), IgG, IgM, IgE, IgA - depleted, Pooled	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), IgG-depleted, Individual	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), IgG, IgM, IgE, IgA - depleted, Individual	HPL-BL
Serum Pharmacokinetic Reasearch System (Human Serum), IgG-depleted, Pooled	HSE-PL
Serum Pharmacokinetic Reasearch System (Human Serum), IgG, IgM, IgE, IgA - depleted, Pooled	HSE-PL
Serum Pharmacokinetic Reasearch System (Human Serum), IgG-depleted, Pooled	HSE-PL

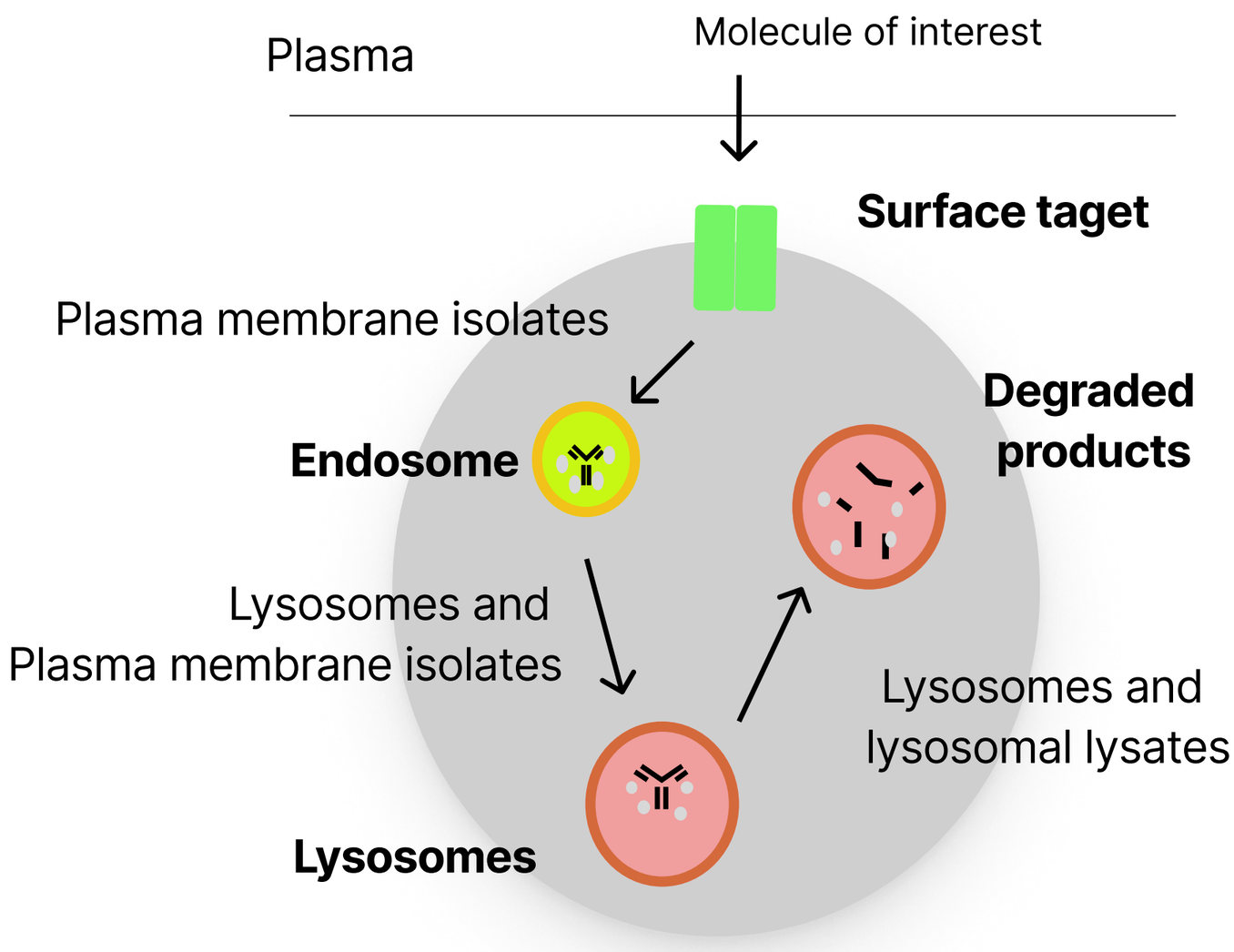
Products in stock	Product code
Serum Pharmacokinetic Reasearch System (Human Serum), IgG, IgM, IgE, IgA - depleted, Pooled	HSE-PL
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), K2EDTA, Pooled	WBL
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), Li Heparin, Pooled	WBL-LIH
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), Na Heparin, Pooled	WBL-NAH
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), K3EDTA, Pooled	WBL-K3
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), K2EDTA, Individual	WBL
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), Li Heparin, Individual	WBL-LIH
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), Na Heparin, Individual	WBL-NAH
Whole Blood Pharmacokinetic Reasearch System (Human Whole Blood), K3EDTA, Individual	WBL-K3
Plasma Pharmacokinetic Research System (Human Plasma), K2EDTA, Pooled	HPL-BL
Plasma Pharmacokinetic Research System (Human Plasma), Li Heparin, Pooled	HPL-LIH
Plasma Pharmacokinetic Research System (Human Plasma), Na Heparin, Pooled	HPL-NAH

Products in stock	Product code
Plasma Pharmacokinetic Research System (Human Plasma), K3EDTA, Pooled	HPL-K3
Plasma Pharmacokinetic Research System (Human Plasma), Hemolyzed, Pooled	HPL-HEM
Plasma Pharmacokinetic Research System (Human Plasma), Lipemic, Pooled	HPL-LIP

3.3. Metabolism in Tissues

On-target and off-target anibody binding frequently leads to the increased metabolism and low free drug availability, especially in case of ADCs and Oligonucletides. TCR tissue set and assays with frozen tissues provides the general picture of the probability of the undesired drug accumulation off-target. However, it has limited predictive capacity in terms of metabolic rate. Since the binding is the reversible process and clearance is irreversible, prediction of the clearance value is highly important for estimating human dose and adverse effect levels.

S9 and lysosomes has better predictive power in terms of compound metabolism, in context of proteolytic cleavage and nuclease activity. We have designed the comprehensive lysosomal panel for drug accumulation and clearance studies.



Antibody–drug conjugates (ADCs) and oligonucleotide therapeutics are both internalized into cells via receptor-mediated endocytosis, followed by trafficking through early and late endosomes to lysosomes. In this acidic, enzyme-rich environment, proteases and glycosidases degrade the conjugate or carrier component. For ADCs, this leads to cleavage of the linker and release of the cytotoxic payload into the cytosol, where it reaches its molecular target. The rate and efficiency of this process depend strongly on the linker design and lysosomal activity.

Oligonucleotides follow a similar route, where lysosomal enzymes gradually degrade unprotected regions, while backbone and sugar modifications enhance stability. Some molecules escape the lysosome and reach the cytosol or nucleus to interact with RNA targets through RNase H or RNA interference mechanisms. Thus, lysosomal metabolism and endosomal trafficking are central to the intracellular activation and pharmacokinetic behavior of both ADCs and oligonucleotide drugs.

The panel of human lysosomal fractions derived from intestine, lung, brain, tumor, muscle, liver, kidney, and spleen enables comprehensive assessment of tissue-specific degradation routes and off-target cleavage of macromolecular therapeutics such as ADCs and oligonucleotides. Since lysosomal catabolism determines intracellular payload release and contributes to systemic clearance, studying tissue variability is essential for predicting unwanted accumulation or degradation outside the target site. Liver, kidney, and spleen lysosomes are particularly relevant for evaluating systemic disposition and immune-related uptake, while intestinal, lung, and muscle lysosomes provide insight into extrahepatic exposure and potential tissue-specific degradation. Tumor and brain lysosomes allow focused investigation of selective activation and CNS stability, respectively. To characterize these processes, we quantify nucleic acid hydrolysis using FRET-based probes and measure proteolytic activity through cathepsin B and L assays, alongside β-N-acetylglucosaminidase (NAG) activity, providing a detailed enzymatic profile for each lysosomal preparation.

Products in stock	**Per request products**
Human Intestinal Lysosomes Human Lung Lysosomes Human Brain Lysosomes Human Tumor Lysosomes Human Muscle Lysosomes Human Liver Lysosomes Human Kidney Lysosomes Human Spleen Lysosomes	Lysosomal lysates, dialized (7kDa) against 0,001M Tris Animal tritosomes Activity probes and assay kits Plasma membrane preparations from the same organs

Lysosomal lysates dialyzed against 0.001 M Tris provide a highly defined biochemical matrix for studying intracellular degradation pathways of biologics and conjugated drugs. Since cumbersome macromolecules cannot penetrate intact lysosomes, the dialyzed lysates enable direct access of substrates to the active enzymes, allowing precise kinetic measurements of degradation without the limitations of membrane permeability.

Animal tritosomes complement these human materials by offering scalable, reproducible systems for comparative or mechanistic studies of lysosomal degradation under standardized enzymatic conditions. Activity probes and assay kits enable quantitative characterization of specific enzymatic pathways—such as cathepsin- or glycosidase-driven cleavage—allowing precise monitoring of drug or linker stability. Plasma membrane preparations from the same organs complete the profiling toolkit by distinguishing lysosomal degradation from surface uptake and transport phenomena, supporting a full understanding of intracellular trafficking, off-target effects, and tissue-specific distribution of macromolecular therapeutics.

5. Catalogue of cellular products

Product Name	Cat No	Price,USD	Stock
Pooled Human Hepatocytes (20-donor pool)	HEP-S-20	550	Stock
Pooled Human Hepatocytes (10-donor pool)	HEP-S-10	500	Stock
Induction-Qualified Plateable Hepatocytes	HEP-P	660	Stock
Human Pooled-5 Plateable Hepatocytes	HEP-P-5	700	Stock
Metabolic Disease-derived Plateable Hepatocytes	HEP-P	700	Stock
Human Liver Sinusoidal Endothelial Cells	LSEC	550	Stock
Human Liver Stellates	STE	650	Stock
Human Kupffer Cells	KUP	600	Stock
CD-1 Mouse Hepatocytes	HEP-CD1	288	Stock
SD Rat Hepatocytes	HEP-SD	288	Stock
Beagle Dog Hepatocytes	HEP-BD	330	Stock
Cynomolgus Monkey Hepatocytes	HEP-CM	438	Stock
Human Suspension Enterocytes	INT	500	Stock
Human Pooled Suspension Enterocytes	ENT-P	550	Stock
Human Plateable Enterocytes	ENT	700	Stock
Human Small Airway Epithelial Cells	SAEC	700	Stock
Human Primary Bronchial Cells	BEC	700	Stock
Human Alveolar Epithelial Cells	AEC	700	Stock
Human Primary Keratinocytes	KER	680	Stock
Human Primary Dermal Fibroblasts	FIB	500	Stock

5. Catalogue of cellular products

Product Name	Cat No	Price,USD	Stock
Human Brain Microvascular Endothelial Cells	HBMEC	680	Stock
Human Brain Astrocytes	AST	800	Stock
Human Brain Pericytes	PER	800	Stock
Primary Human Proximal Tubular Epithelial Cells (PTECs)	PTEC	550	Stock
Primary Human Podocytes	POD	550	Per request
Human Umbilical Cord Endothelial Cells	HUVEC	250	Stock
Human Brain Microvascular Endothelial Cells	HBMEC	680	Stock
Human Liver Sinusoidal Endothelial Cells	LSEC	550	Stock

5. Catalogue of subcellular products

Product Name	Cat No	Price,USD	Stock
NASH-Derived Human Liver S9 Fraction (Pool-20)	S9-H50	90	Per request
Human Liver Microsomes	MIC-H50	100	Stock
Human Liver S9 fraction (Pool-50)	S9-H50	90	Stock
Human Liver Lysosomes	LYS-LIN	245	Stock
Human Liver Homogenate	HMG-LIN	73	Stock
CD-1 Mouse Microsomes	MIC-CD1	49	Stock
SD Rat Microsomes	MIC-SD	49	Stock
Beagle Dog Microsomes	MIC-BD	80	Stock
Cynomolgus Monkey Microsomes	MIC-CM	300	Stock
Human Intestinal S9 Fraction	S9-INT	39	Stock
Human Intestinal Lysosomes	LYS-INT	310	Per request
Human Intestinal Microsomes	MIC-INT	116	Stock
Human Intestinal Homogenate	HMG-INT	100	Stock
Human Lung S9 Fraction	S9-LUN	65	Stock
Human Lung Lysosomes	LYS-LUN	300	Per request
Human Lung Microsomes	MIC-LUN	80	Stock
Human Lung Homogenate	HMG-LUN	120	Stock

5. Catalogue of subcellular products

Product Name	Cat No	Price,USD	Stock
Human Kidney S9 Fraction	S9-KDN	54	Stock
Human Kidney Microsomes	MIC-KDN	104	Stock
Human Kidney Lysosomes	LYS-KDN	280	Stock
Human Kidney Homogenate	HMG-KDN	60	Stock
Human Skin Microsomes	MIC-SKN	180	Stock
Human Skin S9 Fraction	S9-SKN	149	Stock
Human Skin Homogenate	HMG-SKN	90	Stock
Human Frozen Skin Disc	DISC	600	Per request
Human Brain Homogenate	HMG-BRN	184	Stock
Human Brain Mitochondria	MIT-BRN	300	Per request
Human Brain Lysosomes	LYS-BRN	350	Per request
Human Spleen Homogenate	HMG-SPL	60	Stock
Human Spleen Lysosomes	LYS-SPL	280	Stock
Human Muscle Mitochondria	MIT-MSL	280	Stock
Human Muscle Homogenate	HMG-MSL	120	Stock
Human Muscle Lysosomes	LYS-MSL	280	Per request
Human Colorectal Cancer Lysosomes	LYS	280	Stock
Human Breast Cancer Lysosomes	LYS	280	Stock
Human Lung Cancer Lysosomes	LYS	280	Stock

Annex A: Hepatocytes thawing and assay launch

Materials and Equipment:

Water bath with heating
Serological pipettes
Micropipettes
Centrifuge with rotor for 50 mL tubes
+37 incubator with 5% CO2
Laminar Box
Sterile Box with ice
Liquid Nitrogen tank
Counting chamber
Collagen 1 coated 24- or 96-well plates

Reagents:

William's E Medium (Thermo Fisher)
Fetal Bovine Serum (Sigma)
Bovine insulin (Sigma)
HepExtend Supplement 50X (Thermo Fisher)
Percoll (Cytiva)
Antibiotic/Antimycotic 100X (Thermo Fisher)
Trypan Blue Solution (Thermo Fisher)

Caution!

It is critical to store obtainedcells in a temperature lower than -150oC.
DO NOT store cells in the dry ice. Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Reagent Preparation

Prepare Thawing and Culture Medium: Mix RPMI 1640 Medium with Fetal Bovine Serum: final concentration 5% for Thawing Medium and 10% for Culture Medium, add GlutaMax to Culture Medium according to manufacturer protocol, and Antibiotic-Antimycotic or Penicillin/Streptomycin solution to final concentration 1%.
All media should be stored at 4 °C until use. The volume of Thawing Medium for 1 vial of Kupffer Cells is 10 mL.

Reagent Preparation

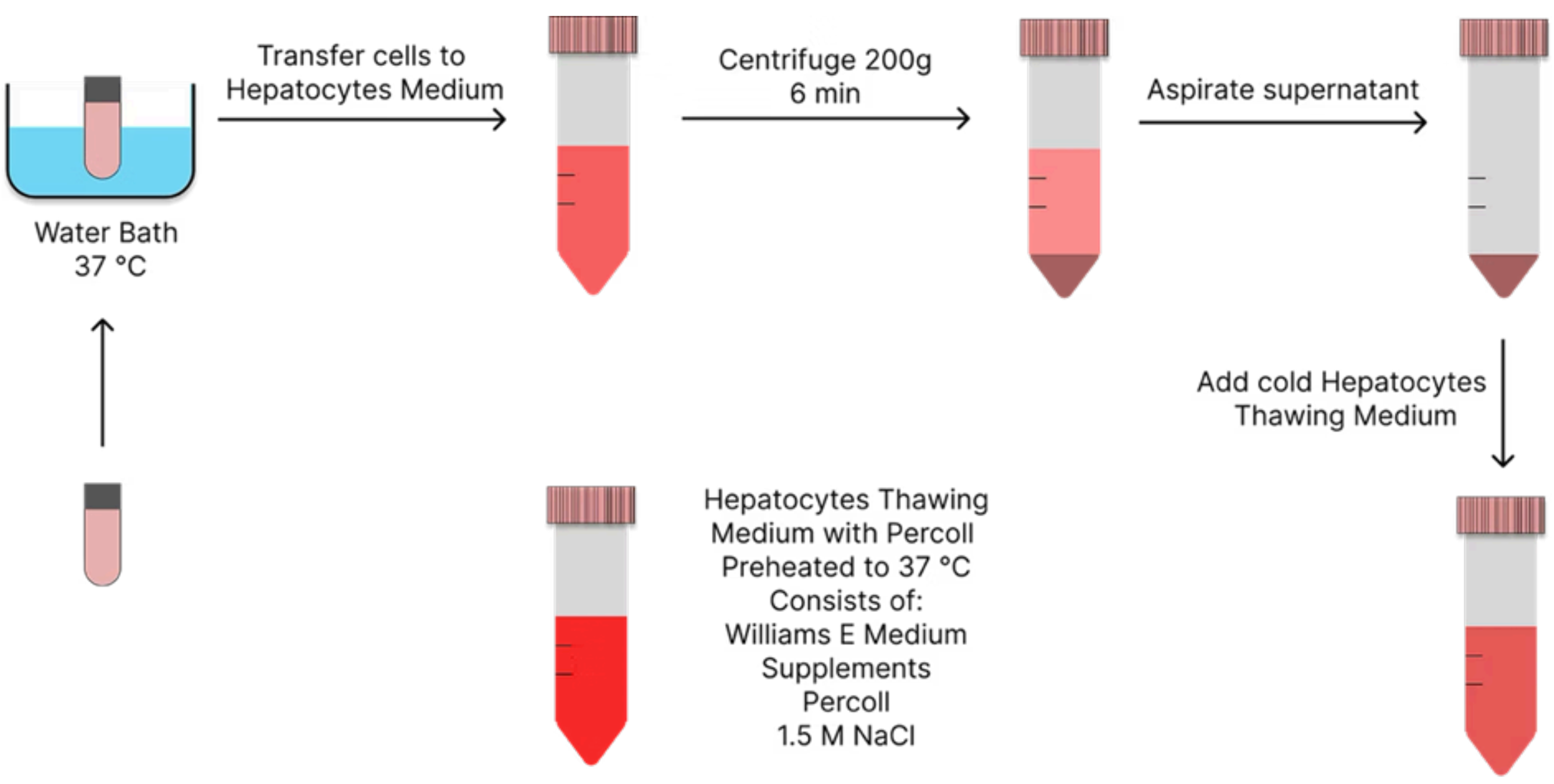
Prepare Hepatocyte Medium: Mix William's E Medium (450 mL) with 50 mL of Fetal Bovine Serum (final concentration 10%), add Bovine insulin (4 µg/mL) and Antibiotic-Antimycotic solution to final concentration 1%.

Note: HepExtend Supplement could be used as serum-free alternative to FBS and Bovine insulin for preparation of Hepatocyte Medium. The Medium should be stored at 4 °C until use.

Dilute 5.04 ml of Percoll with 0.56 ml of 1.5 M NaCl water solution.

Prepare Hepatocyte Thawing Medium by adding Percoll solution to Hepatocyte Medium.

The volume of Thawing Medium for 1 vial of Plateable Hepatocytes is 20 mL: Add 5,6 mL of Percoll solution to 14,4 mL of Hepatocyte Medium and mix well. The Thawing Medium should be preheated to 37 °C before thawing procedure.



Thawing of Hepatocytes

Remove the required number of cryovials with frozen cells from the liquid nitrogen tank.

Quickly place the cryovials in a 37°C water bath (do not use an incubator). Slightly open and then close the vial to remove the excessive Liquid Nitrogen.

While holding the tip of the bottle, mix gently, not allowing water to penetrate through the cap.
DO NOT submerge the cryotube completely in water.

Thaw the vial of cells in a water bath until half of the ice pellet remains (this usually takes about 80-120 seconds).

Immediately remove the vial from the water bath. Wipe the outside of the bottle with an alcohol wipe. Place the thawed vial in the laminar box.

Gently transfer the contents of the cryovial to preheated Hepatocyte Thawing Medium.

Carefully rinse the cryotube with 1 ml of Thawing medium and transfer the remaining cell suspension to a vial. Repeat this step 2-3 times.

Gently mix the cell suspension in the vial (DO NOT vortex)

Centrifuge at 200g/ 6 min/ at room temperature.

Carefully aspirate the supernatant without disturbing the cell pellet.

Resuspend the cell pellet in 3-4 ml of cold Hepatocyte Medium.
DO NOT use small tips to resuspend cells, it's better to use 5 mL pipette for more gentle resuspending of hepatocyte pellet.

Measure the exact volume of the cell suspension with a 5 mL graduated pipette. Cell suspension should be stored on ice or at +4°C during all next procedures.

Check cell concentration and viability. For in-process control as well as quality control of the end product, cell count and viability could be determined by light microscopy and trypan blue exclusion.
EAPH are thawed using the standard protocol for primary hepatocytes and fractionated with Percoll (28-30%)

Thawed EAPH are diluted to the desired concentration in incubation medium and added to the wells

The test compound is added to each well to achieve a final concentration of 1 µM

The plate is placed in an incubator (37 °C and 5% CO2) on an orbital shaker (100–150 rpm).

Incubation solutions are then quenched with acetonitrile and centrifuged, then the supernatants are collected and analyzed for the remaining test compound using a suitable method (e.g., chromatography).

The in vitro half-life (t1/2) is determined for the parent compound by regression analysis of the compound consumption vs. time.

Annex B: Kupffer cells thawing and culturing

Materials and Equipment:

Water bath with heating
Serological pipettes
Micropipettes
Centrifuge with rotor for 50 mL tubes
+37 incubator with 5% CO2
Laminar Box
Sterile Box with ice
Liquid Nitrogentank
Counting chamber
Collagen 1 coated flasks orplates

Reagents:

RPMI 1640 Medium (Thermo Fisher)
Fetal Bovine Serum (Sigma)
GlutaMax (Gibco)
Antibiotic/Antimycotic or Penicillin/Streptomycin (Thermo Fisher)
Trypan Blue Solution (Thermo Fisher)

Caution!

It is critical to store obtainedcells in a temperature lower than -150oC.
DO NOT store cells in the dry ice. Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Reagent Preparation

Prepare Thawing and Culture Medium: Mix RPMI 1640 Medium with Fetal Bovine Serum: final concentration 5% for Thawing Medium and 10% for Culture Medium, add GlutaMax to Culture Medium according to manufacturer protocol, and Antibiotic-Antimycotic or Penicillin/Streptomycin solution to final concentration 1%.

All media should be stored at 4 °C until use. The volume of Thawing Medium for 1 vial of Kupffer Cells is 10 mL.

Thawing of Kupffer Cells

Remove the required number of cryovials with frozen cells from the liquid nitrogen tank.

Quickly place the cryovials in a 37°C water bath (do not use an incubator). Slightly open and then close the vial to remove the excessive Liquid Nitrogen.

While holding the tip of the vial, mix gently, not allowing water to penetrate through the cap.
DO NOT submerge the cryotube completely in water.

Thaw the vial of cells in a water bath until half of the ice pellet remains (this usually takes about 60-90 seconds).

Immediately remove the vial from the water bath. Wipe the outside of the vial with an alcohol wipe. Place the thawed vial in the laminar box.

Transfer the contents of cryovial into a 50-mL conical tube containing 10 mL of cold (4°C) Thawing Medium. Rinse the vial few times with Thawing Medium.

Note: Kupffer cells are very "sticky" at physiological temperature of 37°C. If the medium is warmed to 37°C, the Kupffer cells will attach to any substrate including the walls of the conical tube. Therefore, the use of pre-warmed media is not recommended at this step.

Centrifuge the cells at 600 × g for 6 minutes at +4 oC.
Note: It is recommended to hold the cells on ice until plating.

Resuspend the pelleted cells (note that the pellet will be very small) in 1–2 mL of Culture Medium pre-cooled to +4 oC using a micropipette. Serological pipette can also be used however this may lead to clumping of the cells.

Count the cells using the trypan blue exclusion assay in Counting Chamber.

Plating and Cultivation of Kupffer Cells

For Plating and Cultivation of cells Culture Medium should be used.

The cells should be seeded on Collagen 1 coated plates or flasks. Depending on area, the number and concentration of seeded cells varies (0.2 × 10^6 to 0.4 × 10^6 cells/mL).

After plating of Kupffer Cells, place them in a humidified 37°C/5% CO2 incubator and allow to attach for 6 hours.

After 6 hours of attachment, replacethe medium with fresh Culture Medium.

After 24 hours, replace the medium with fresh Culture Medium again and proceed with experiment.

Note: To maintain Kupffer cell cultures for longer (1–2 weeks), replace medium with fresh Culture Medium every 24–48 hours.

Annex C: Lung cells thawing and culture

Materials and Equipment:

Water bath with heating
Serological pipettes
Micropipettes
Centrifuge with rotor for 50 mL tubes
+37 incubator with 5% CO2
Laminar Box
Sterile Box with ice
Liquid Nitrogen tank
Counting chamber
Collagen 1 coated flasks or plates

Reagents:

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Thermo Fisher)
Fetal Bovine Serum (Sigma-Aldrich)
Glutamax (Gibco)
EGF (Sigma-Aldrich)
Bovine insulin (Thermo Fisher)
Transferrin (Sigma-Aldrich)
Hydrocortisone (Sigma-Aldrich)
Epinephrine (Sigma-Aldrich)
Antibiotic/Antimycotic or Penicillin/Streptomycin (Thermo Fisher)
Trypan Blue Solution (Thermo Fisher)
ROCK Inhibitor Y-27632

Caution!

It is critical to store obtainedcells in a temperature lower than -150oC.
DO NOT store cells in the dry ice. Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Reagent Preparation

Prepare Thawing and Culture Medium for Lung Epithelial Cells: Mix DMEM:F-12 Medium with Fetal Bovine Serum: final concentration 10% for Thawing Medium and 2% for Culture Medium;

Add Growth Factors and Hormones to Culture Medium: EGF (10 ng/mL); Insulin (5 µg/mL); Transferrin (10 µg/mL); Hydrocortisone (0,5 µg/mL); Epinephrine (0,5 µg/mL);

For the first 24–48 hours after seeding, supplement the culture medium with ROCK inhibitor (10 µM) to enhance cell attachment and survival.

Add Antibiotic-Antimycotic or Penicillin/Streptomycin solution to Thawing and Culture Medium in final concentration 1%.

Warm all media to 37°C before use.

Thawing of Lung Epithelial Cells

Remove the required number of cryovials with frozen cells from the liquid nitrogen tank.

Quickly place the cryovials in a 37°C water bath (do not use an incubator). Slightly open and then close the vial to remove the excessive Liquid Nitrogen.

While holding the tip of the vial, mix gently, not allowing water to penetrate through the cap.
DO NOT submerge the cryotube completely in water.

Thaw the vial of cells in a water bath until half of the ice pellet remains (this usually takes about 60-90 seconds).
Immediately remove the vial from the water bath. Wipe the outside of the vial with an alcohol wipe. Place the thawed vial in the laminar box.

Transfer the contents of cryovial into a 50-mL conical tube containing 10 mL (37°C) of Lung Epithelial Cells Thawing Medium. Rinse the vial a few times with Thawing Medium.

Centrifuge the cells at 300 × g for 10 minutes.

Resuspend the pelleted cells in 1-2 mL of Lung Epithelial Cells Culture Medium using a micropipette or serological pipette.
Count the cells using the trypan blue exclusion assay in Counting Chamber.

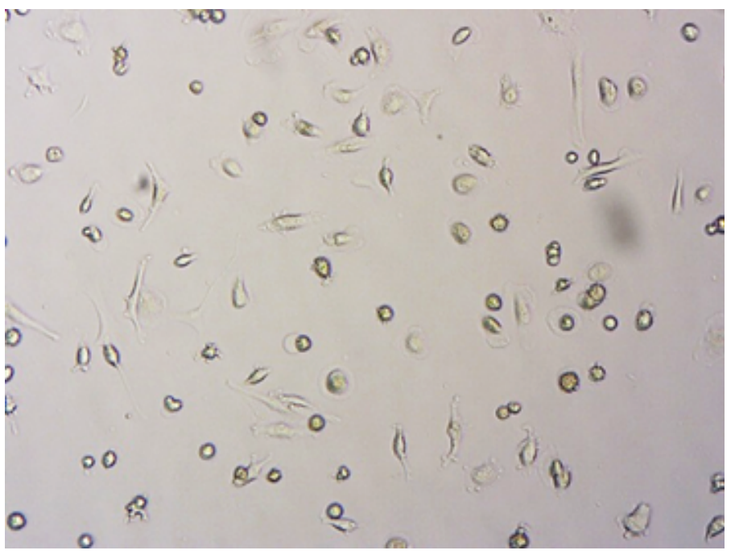
Plating and Cultivation of Lung Epithelial Cells

For Plating and Cultivation of cells Culture Medium should be used.
Lung Epithelial Cells should be seeded on Collagen 1 coated plates or flasks.

A seeding density of 10,000-15,000 cells/cm2 is recommended.

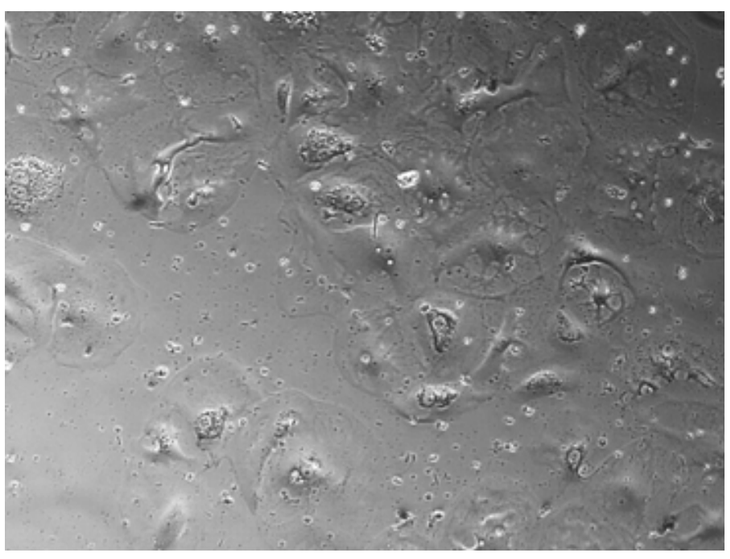
After plating cells in optimal seeding density, place them in a humidified 37°C/5% CO2 incubator and allow to attach for at least 16 hours.

Next day after seeding replace the medium with fresh Culture Medium to remove unattached cells.
Change the medium every two days thereafter, until the culture is approximately 70-80% confluent.

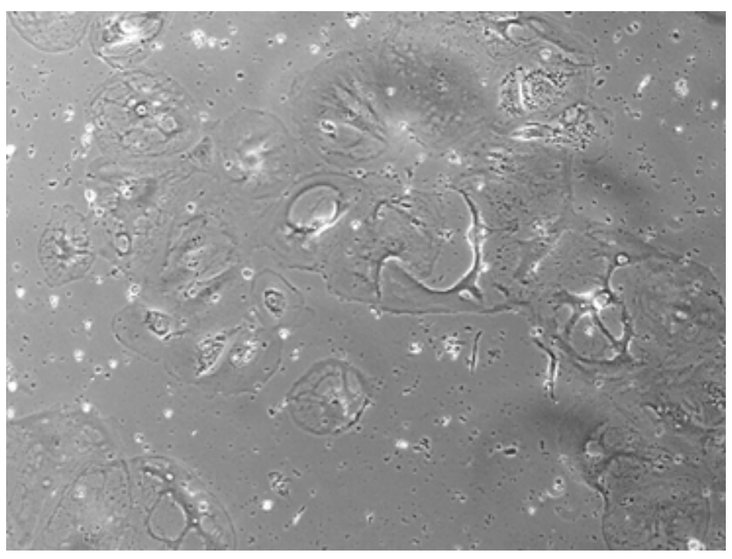


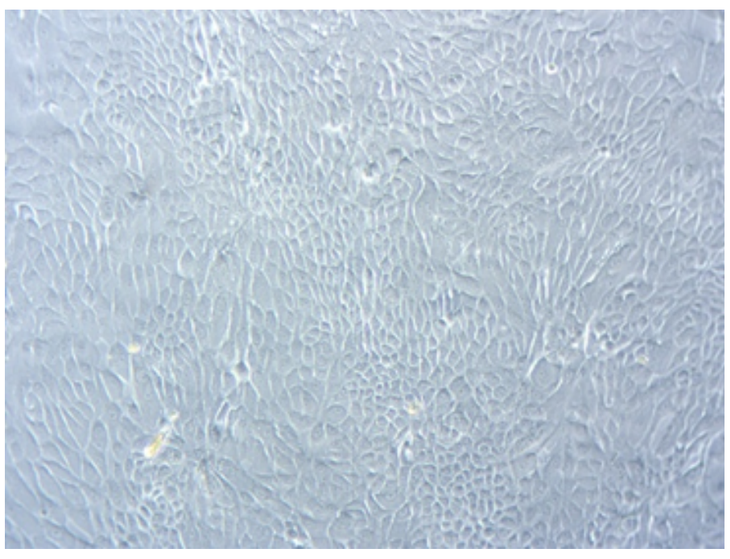
AEC on the day after seeding

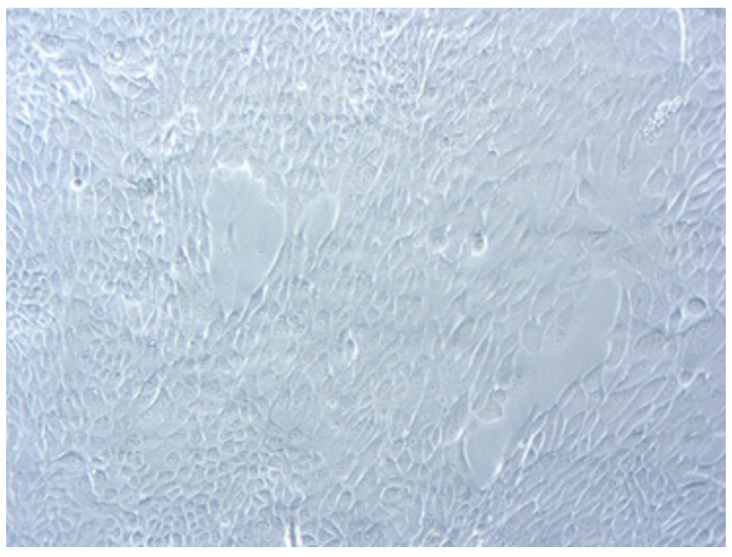
AEC after 2 weeks growing in culture



BEC after 5-8 days growing in culture







SAEC after 2-3 weeks growing in culture

Annex D: Intestinal cells culture in 2D

Materials and Equipment:

Water bath with heating
Serological pipettes
Micropipettes
Centrifuge with rotor for 50 mL tubes
+37 incubator with 5% CO2
Laminar Box
Sterile Box with ice
Liquid Nitrogen tank
Counting chamber
Gelatin-coated flasks or plates

Reagents:

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Thermo Fisher)
Fetal Bovine Serum (Sigma-Aldrich)
Antibiotic/Antimycotic or Penicillin/Streptomycin (Thermo Fisher)
Intestinal Epithelial Cell Medium (TM090, Applied Biological Materials)
Trypan Blue Solution (Thermo Fisher)
ROCK Inhibitor Y-27632 (STEMCELL)

Caution!

It is critical to store obtainedcells in a temperature lower than -150oC.
DO NOT store cells in the dry ice. Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Reagent Preparation

Prepare Thawing Medium: Mix DMEM:F-12 Medium with Fetal Bovine Serum: final concentration 10% for Thawing Medium.
Add Antibiotic-Antimycotic or Penicillin/Streptomycin solution to Thawing Medium in final concentration 1%.
Culture medium in this formulation is ready-to-use.
For the first 24–48 hours after seeding, supplement the culture medium with ROCK inhibitor (10 μM) to enhance cell attachment and survival.
Warm all media to 37°C before use.

Thawing of Intestinal Cells and Enterocytes

Remove the required number of cryovials with frozen cells from the liquid nitrogen tank.

Quickly place the cryovials in a 37°C water bath (do not use an incubator). Slightly open and then close the vial to remove the excessive Liquid Nitrogen.

While holding the tip of the vial, mix gently, not allowing water to penetrate through the cap.
DO NOT submerge the cryotube completely in water.

Thaw the vial of cells in a water bath until half of the ice pellet remains (this usually takes about 60-90 seconds).

Immediately remove the vial from the water bath. Wipe the outside of the vial with an alcohol wipe. Place the thawed vial in the laminar box.

Transfer the contents of cryovial into a 50-mL conical tube containing 10 mL (37°C) of Thawing Medium. Rinse the vial a few times with Thawing Medium.

Centrifuge the cells at 300 × g for 10 minutes.

Remove supernatant carefully by aspiration without disturbing the cell pellet.

Re-suspend the cells by adding 1-2 ml of Culture Medium to the cell pellet. Evenly re-suspend the pellet to no visible cell clumps can be seen.
Count the cells using the trypan blue exclusion assay in Counting Chamber.

Plating and Cultivation of Intestinal Cells and Enterocytes

For Plating and Cultivation of cells Culture Medium should be used.

The cells should be seeded on Gelatin-coated (1% gelatin solution) plates or flasks.
Re-suspend the cells to seeding density of 4 x 105 /mL and seed on flask or plate for cultivation.

After plating of cells, place them in a humidified 37°C/5% CO2 incubator and allow to attach for at least 24 hours.

Next day after seeding replace the medium with fresh Culture Medium to remove unattached cells.

Change the Culture Medium 2-3 days thereafter.

Annex E: Stellate cells and LSECs thawing and culture

Materials and Equipment:

Water bath with heating
Serological pipettes
Micropipettes
Centrifuge with rotor for 50 mL tubes
+37 incubator with 5% CO2
Laminar Box
Sterile Box with ice
Liquid Nitrogen tank
Counting chamber
Collagen 1 coated flasks or plates
Tissue culture treated flasks or plates

Reagents:

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Thermo Fisher)
DMEM with High Glucose (Sigma-Aldrich)
Fetal Bovine Serum (Sigma-Aldrich)
VEGF (Sigma-Aldrich)
EGF (Sigma-Aldrich)
bFGF (Sigma-Aldrich)
Bovine insulin (Thermo Fisher)
Heparin (Sigma-Aldrich)
Dexamethasone (Sigma-Aldrich)
Antibiotic/Antimycotic or Penicillin/Streptomycin (Thermo Fisher)
Trypan Blue Solution (Thermo Fisher)
ROCK Inhibitor Y-27632 (STEMCELL)

Caution!

It is critical to store obtained cells in a temperature lower than -150oC.
DO NOT store cells in the dry ice. Use personal protective equipment: lab coat, gloves and goggles when working with cell cultures.

Reagent Preparation

Prepare Thawing and Culture Medium for LSECs: Mix DMEM:F-12 Medium with Fetal Bovine Serum: final concentration 10% for Thawing Medium and 5% for Culture Medium;

Add Growth Factors and Hormones to Culture Medium: VEGF (25 ng/mL); bFGF (10 ng/mL); EGF (10 ng/mL); Insulin (5 µg/mL); Heparin (50 µg/mL); Dexamethasone (10 µM);

Add Antibiotic-Antimycotic or Penicillin/Streptomycin solution to Thawing and Culture Medium in final concentration 1%.

Prepare Thawing and Culture Medium for HSCs: Mix DMEM Medium with High Glucose with Fetal Bovine Serum: final concentration 10% for Thawing Medium and 10% for Culture Medium

Add Antibiotic-Antimycotic or Penicillin/Streptomycin solution to final concentration 1%.
For the first 24–48 hours after seeding, supplement the culture medium with ROCK inhibitor (10 µM) to enhance cell attachment and survival.

Warm all media to 37°C before use.

Thawing of Lung Epithelial Cells

Remove the required number of cryovials with frozen cells from the liquid nitrogen tank.

Quickly place the cryovials in a 37°C water bath (do not use an incubator). Slightly open and then close the vial to remove the excessive Liquid Nitrogen.

While holding the tip of the vial, mix gently, not allowing water to penetrate through the cap.
DO NOT submerge the cryotube completely in water.

Thaw the vial of cells in a water bath until half of the ice pellet remains (this usually takes about 60-90 seconds).

Immediately remove the vial from the water bath. Wipe the outside of the vial with an alcohol wipe. Place the thawed vial in the laminar box.

Transfer the contents of cryovial into a 50-mL conical tube containing 10 mL (37°C) of LSEC or HSteC Thawing Medium. Rinse the vial a few times with Thawing Medium.

Centrifuge the cells at 300 × g for 6 minutes.

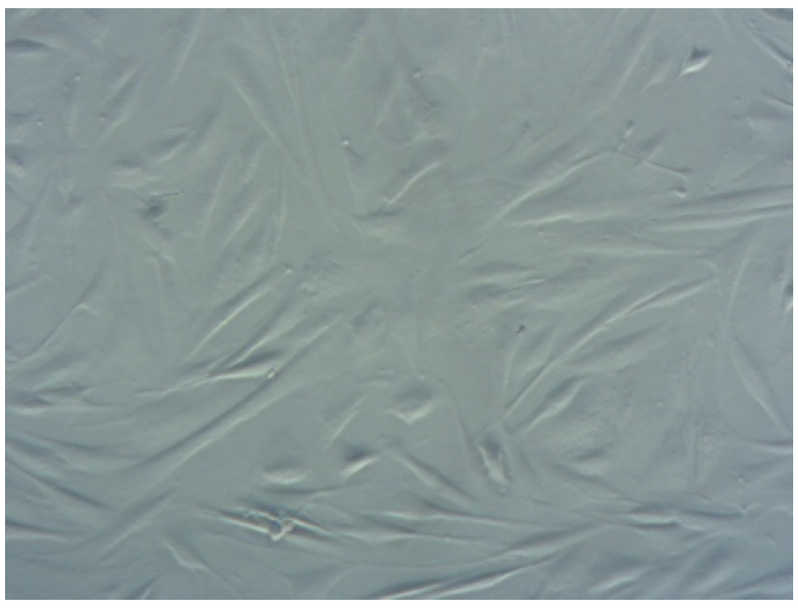
Resuspend the pelleted cells (note that the pellet will be very small) in 1-2 mL of LSEC or HSteC Culture Medium using a micropipette or serological pipette.
Count the cells using the trypan blue exclusion assay in Counting Chamber.

Plating and Cultivation of Lung Epithelial Cells
For Plating and Cultivation of cells Culture Medium should be used.

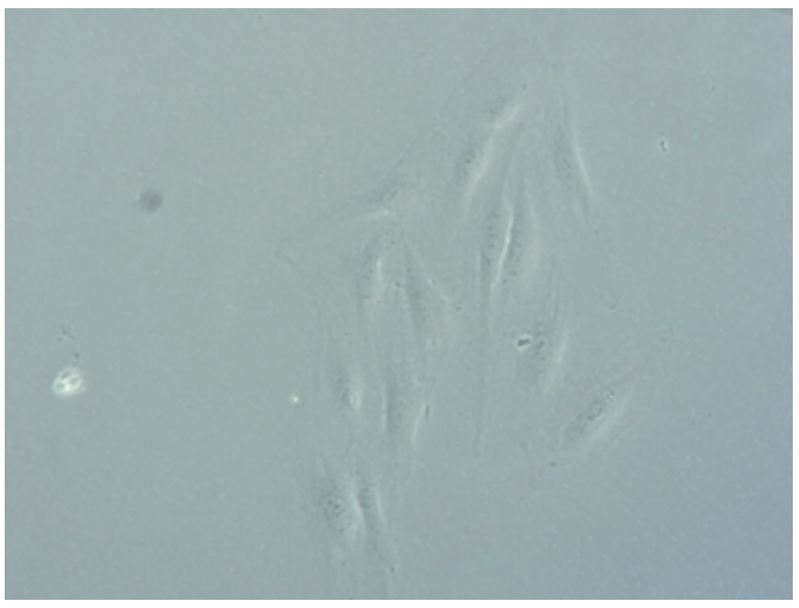
The LSECs should be seeded on Collagen 1 coated plates or flasks, and HSteCs could be seeded on tissue culture treated flasks.

Inoculate at 15,000 cells per cm2 for rapid growth, or at 10,000 cells per cm2 for regular subculturing of LSECs. Optimal seeding density of HSteCs is 10,000-15,000 cells per cm2.
After plating of cells, place them in a humidified 37°C/5% CO2 incubator and allow to attach for at least 16 hours.

Next day after seeding replace the medium with fresh Culture Medium to remove unattached cells. Change the medium every two days thereafter, until the culture is approximately 70% confluent.
Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.



Stellate Cells after reaching confluence



LSEC after reaching confluence