

## User Guide

## Normal Human Hepatic Stellate Cells

**HLP301-100K, HLP301-200K, HLP301-250K, HLP302-1M****Store in Liquid Nitrogen.****FOR RESEARCH USE ONLY****Not for Use in Diagnostic Procedures. Not for Human or Animal Consumption.**

### Product Overview

Primary Human Stellate cells are isolated from whole human livers that are deemed not suitable for liver transplantation and have received consent to be donated for research. Hepatic Stellate Cells (HSCs; also called as vitamin A-storing cells, lipocytes, interstitial cells, fat-storing cells, Ito cells) exist in the space between Parenchymal cells and Sinusoidal Endothelial cells of the hepatic lobule. Stellate cells store 80% of vitamin A in the whole body as retinyl palmitate in lipid droplets in the cytoplasm and are responsible for Vitamin A homeostasis. Stellate cells have a star-like appearance and upon activation can acquire contractile myofibroblast-like morphology which is an essential step in fibrosis. In fibrosis, activated stellate cells display a loss of stored vitamin A, and increased collagen synthesis along with other extracellular matrix components. Each lot is guaranteed for post-thaw cell viability of  $\geq 70\%$ . All the lot-specific information including donor information can be obtained via Certificate of Analysis (CoA) upon request.

### Quality Control Testing

- Post-thaw viability  $\geq 70\%$ , with a yield of  $\geq 100K$ , 200K, 250K, or 1M viable cells per vial.
- Cell marker analysis:  $\alpha$ SMA, Vimentin and Vinculin.
- Each donor is tested negative for: HIV, Hepatitis B, Hepatitis C, and syphilis\*.
- The culture is tested negative for: Gram +, Gram –, Mycoplasma and Fungi.

\*No known test can offer complete assurance that the viruses that cause HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C are not present. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2) or higher.

### Materials Provided

Normal Human Hepatic Stellate Cells:

One (1) vial containing  $\geq 100K$ , 200K, 250K or 1M cells.

## Materials Required (Not Provided)

- Collagen Type I, Rat Tail (Cat No. #08-115).
- Tissue culture treated Multiwell plates.
- Trypsin solution, 0.25% with EDTA (Cat No. #T4049).

Please see [Protocols](#) for media components.

## Storage

Upon receipt, immediately store cryovial(s) in vapor phase liquid nitrogen.

## Protocols

All protocols are performed within a Class II laminar flow biohood and with an aspirator unless otherwise specified. Incubators are humidified and are set to 37 °C and 5% CO<sub>2</sub>.

PPE should be worn such as gloves, lab coat, and safety glasses.

### Preparing Collagen Coated Plate

1. Dilute the collagen to a final concentration of 56 g/mL in sterile 70% ethanol and gently mix until the collagen is solubilized.
2. Add the appropriate volume of the collagen/ethanol mixture to each well to completely cover the bottom of wells.
3. Gently move the cell culture plate until the collagen/ethanol mixture evenly coats the inside of the well.
4. Air dry plates in a laminar flow hood. Leave cell culture plate over night with the cover ajar to allow airflow and prevent condensation.

### Preparing Stellate Medium for Human Hepatic Stellate Cells

Formulations for human hepatic stellate cell media are readily available from literature. Below is an example media from one of the publications\*. All components listed below are available at [SigmaAldrich.com](https://www.sigmaaldrich.com).

| Components                  | Cat No.     | Working Stock | Final Dilution | Final Concentration | Final Volume (mL) |
|-----------------------------|-------------|---------------|----------------|---------------------|-------------------|
| DMEM. High Glucose          | D5796-500ML | -             | -              | 1x                  | 445               |
| FBS                         | ES-009-B    | -             | -              | 10%                 | 50                |
| Antibiotic-antimycotic 100x | A5955       | 100x          | 100x           | 1x                  | 5                 |
| Total volume                |             |               |                |                     | 500               |

\* Liu, X., Brenner, D.A., Kisseleva, T. (2023). Human Hepatic Stellate Cells: Isolation and Characterization. In: Weiskirchen, R., Friedman, S.L. (eds) Hepatic Stellate Cells. Methods in Molecular Biology, vol 2669. Humana, New York, NY. [https://doi.org/10.1007/978-1-0716-3207-9\\_13](https://doi.org/10.1007/978-1-0716-3207-9_13)

## Thawing and Plating Stellate Cells

Handle gently and quickly to maintain viability.

Collagen I coated culture ware is required for culturing stellate cells (see above, [Preparing Collagen Coated Plate](#)).

1. Place vial in a 37 °C water bath hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to a sterile work area. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a sterile 15 mL conical tube.
3. Wash vial with warm 5 mL stellate medium and add this wash to conical tube.
4. Centrifuge the tube at 250 x g for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in fresh stellate medium.
5. Perform cell count.
6. For expansion, seed the cells at a density of 4,000 cells/cm<sup>2</sup> on collagen I coated plates.
7. For best results, do not disturb the culture for at least 12 hours after seeding. Change growth medium the next day to remove any residual DMSO or unattached cells, then every other day thereafter.

## Instruction for Sub-Culturing Stellate Cells

1. Subculture cells when they have reached 90% confluency.
2. Warm medium, 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) to room temperature.
3. Aspirate medium, then rinse cells with DPBS. Add trypsin solution into flask and incubate in a 37 °C incubator for 3-5 minutes, or until the cells detach.
4. At the end of trypsinization, wash cells off flask with an appropriate amount of medium.
5. Transfer to centrifuge tube and centrifuge at 250 x g for 5 minutes.
6. After centrifugation, aspirate the medium, re-suspend in 1-2 mL fresh medium and count cells for seeding.
7. Seed the cells at a density of 4,000 cells/cm<sup>2</sup> on collagen I coated plates.

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

## Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

## Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

## Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices).

The life science business of Merck operates  
as MilliporeSigma in the U.S. and Canada.

Merck and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2024-2025 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

23024858 Rev07/25

