

## Primary Human Hepatocytes

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3750	Primary Human Hepatocytes	Donor dependent >1 million cells/ vial	Liquid nitrogen	Liquid nitrogen	See below
ax3751	Primary Hepatocyte Plating Medium	250 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT
ax3752	Primary Hepatocyte Maintenance Medium	250 mL	4°C for 1 month	NA	Thaw at 4°C or RT

Lot-specific information such as donor information is stated in the Certificate of Analysis.

### Recommendations:

- Always count the number of viable cells after thawing
- Recommended culture vessel coating: ax3799, Type I Collagen coated multi-well plates
- Recommended cell culture media: ax3751 Axol Hepatocyte Plating Medium & ax3752 Axol Hepatocyte Maintenance Medium
- Recommended seeding density: 750,000 viable cells/cm<sup>2</sup>
- Recommended centrifugation speed: 100 x g at 4°C for 10 min

### Thawing & Plating:

**Axol Recommends: Always plate Primary Human Hepatocytes on ax3799 (Type 1 Collagen coated multi-well plates)**

- Thaw the cells quickly in a 37°C water bath until just prior to complete thawing.
- Wipe the outside of the vial with 70% ethanol.
- Upon thawing, and for a single cryovial, transfer the cells into a sterile 15 mL conical bottom centrifuge tube at the suggested percoll gradient mixture; see certificate of analysis.

Percoll Gradient	<b>Cold</b> Hepatocyte Plating Medium with cells	Percoll	10x PBS
0%	15 mL	0	0
25%	11.25 mL	3.375 mL	0.375 mL
30%	10.5 mL	4.05 mL	0.45 mL
35%	9.75 mL	4.725 mL	0.525 mL

- Centrifuge the cells at 100 x g at 4°C for 10 min.

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- Carefully remove the supernatant and resuspend in 1-2 mL of **Cold Plating Medium** and perform a cell count.
- Dilute the cells into the required volume of **pre-warmed Plating Medium**.
- Seed cells into the culture vessel at the recommended seeding density.
- Once the cells have attached (after 6-24 h), aspirate the plating media from the cells and replace with **pre-warmed Maintenance Medium**.
- Proceed with experiment assays.

## FAQs

Can I passage the cells?	No. Primary Human Hepatocytes cannot be passaged. Cells should be plated and used directly for endpoint assays.
Does the media contain antibiotics?	Yes.
What is the source of the cells?	Primary Human Hepatocytes are isolated from the whole liver tissue obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation.
What format of the collagen coated culture ware should I use?	Please optimize this for your experiments depending on your end assay.

## Pathogen Testing:

Samples from each donor are tested via PCR to confirm non-reactivity to HIV-1, HIV-2, hepatitis B, hepatitis C and syphilis. However, since no known test can offer complete assurance, please treat the culture as a potentially infectious agent.

## Product Warranty:

Axol Bioscience Ltd. warrants the performance of cells only if the recommended media/reagents are used and the recommended protocols are followed. Cryopreserved cells are assured to be viable when thawed according to the recommended protocol on recommended culture ware.

## Usage Statement:

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

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