NEUROMICS

A Division of CA3 Biosciences, Inc.

# Protocol for Human Hepatocyte

**Characterization of the cells**
Hepatocytes were analyzed morphologically as well as for Albumin Production. Cells test positive for Albumin production. Cells were also tested for active P450 metabolic enzymes. Using LC-MS activity was confirmed for CYP 1A, 1A2, 2B6, 2C19, 2C9, 2D6, and 3A.

**Recommended Products**
Hepatocyte Maintenance Medium (cat# HM42600). Culturing in our Hepatocyte Maintenance Medium will extend the experimental time window the of the hepatocytes for an additional 8 days. Medium changes should be done every other day. Hepatocyte Maintenance Medium contains DMSO, so use nitrile gloves when preparing and changing medium, and discard any unused medium in a closed container as hazardous waste. Hepatocyte Maintenance Medium is light sensitive, so avoid unnecessary exposure to light. To avoid cells drying out media changes, leave a small volume (about 10% of the total volume) of liquid in each well prior to adding fresh medium.

**Handling of Arriving Cells**
Store in liquid nitrogen until ready to culture.

*Note: Handling human derived products is potentially biohazardous. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.*

*Note: Always work under aseptic conditions.*

CULTURE PROTOCOL

1. Prior to starting this experiment, the required plate (96-, 24-, or 12-well) should be properly coated with human type-1 collagen, based on the manufactures' recommendations.
2. Prepare the Medium Thaw Hepatocyte Maintenance Medium at RT. Once thawed, Hepatocyte Maintenance Medium can be stored at 2-8°C for up to three days.
3. Rapidly thaw (<2 minutes) frozen vial of Hepatocytes in a 37°C water bath. Remove vial from water bath just before the last trace of ice has melted. Spray vial with 70% ethanol to prevent contamination in tissue culture hood.
4. **DO NOT** wash cells or spin cells to remove freezing media. DMSO is present in Hepatocyte Maintenance Medium and the residual amounts in the freezing media will not harm the cells.
5. Count cells and plate number as recommended by the plate manufacturer's suggestion.
6. Place cells in 37°C incubator at 5% $CO_2$, replacing media every 2 days.
7. In 4-12 wells at a time, use a pipette to aspirate 90% of the medium from each well, discard, and replace with 0.5 ml/cm2 of warm Hepatocyte Maturation Medium

*Note: Do not allow the cells to dry out during media changes*

8. Incubate the cells at 37°C, 5% $CO_2$, and >90% humidity until the next medium change or until hepatocytes are to be used in experiments.
9. Discard any unused warmed medium.

Note: Should any issues arise while using our cells, our team is here to help troubleshoot any issues. Our cells are backed by our one-time replacement or refund policy. Our recommended protocol including recommended products must be used to be eligible for replacement or refund. Cells that have been refrozen are no longer eligible for refund or replacement.



**www.neuromics.com**

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
Phone 866-350-1500 • fax 612-677-3976 • email: pshuster@neuromics.com