

**BALB/c Primary Hepatocytes - Plateable**

Catalog No. BALB-5224

**Suggested Medium**

M1365 Complete Hepatocyte Medium /w Kit (125 ml)

**Product Description**

BALB/c Primary Hepatocytes from Cell Biologics are isolated from liver tissue of pathogen-free laboratory BALB/c mice. Cells at passage 0 are cryo-preserved and each vial contains  $3 \times 10^6$  cells per ml. Mouse Primary Hepatocytes are characterized by immunofluorescence staining with an antibody of ZO-1. Cells can be plated under cell culture conditions specified by Cell Biologics for the desired experiment. Primary hepatocytes can not be cultured indefinitely and can not be passaged.

**Storage**

Cryopreserved cells will be shipped overnight on dry ice. Upon arrival, please immediately transfer the frozen cells to liquid nitrogen ( $-150^{\circ}\text{C}$ ) until ready for use. Cells should be plated and used directly for the desired study within 1-2 weeks of receipt. Primary cells should never be stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

**Authorized Uses of Cell Biologics' Products**

Primary Hepatocytes from Cell Biologics are distributed for research purposes only. Our products are not authorized for human use, for in vitro diagnostic or therapeutic procedures. Transfer or resale of any Cell Biologics' cells or products from the purchaser to other markets, organizations or individuals is prohibited by Cell Biologics without the company's written consent. Cell Biologics' Terms and Conditions must be accepted before submitting an order.

**Disclaimer**

Investigators should handle the cells with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

**Warranty and Liability**

Cell Biologics' guarantee applies only to your purchase of Cell Biologics' Cells with Cell Biologics' Media and Coating Solution for appropriate cell culture and cell testing following Cell Biologics' online protocols within 35 days from the date of product delivery.

## Primary Hepatocyte Culture Protocol

All cell culture procedures must be performed in a biosafety cabinet.  
All culture media, supplements, and reagents must be sterile filtered through a 0.2 µm filter.  
Use aseptic technique to prevent microbial contamination.

### Medium

Please review the Cell Biologics website for information on appropriate culture media (e.g., serum and other supplements). Use 30-50 mL of cell culture medium (Cat. No. M1365) prewarmed to 37°C for seeding and medium changes.

### Coating of Cell Culture Plates or Dishes

Gelatin coating solution (Product No. CB6950, Cell Biologics) was applied to a sterile dish or flask for 2 min, and the excess solution was aspirated.

### Cell Recovery from Cryovial

1. Thaw the cells quickly (<2 min) in a 37°C water bath until just prior to complete thawing.
2. Wipe the outside of the vial with 70% ethanol.
3. Gently transfer cells (3 million cells/mL) to a sterile 15 mL centrifuge tube.
4. Gently add 7 mL of pre-warmed Cell Biologics cell culture medium (Cat. No. M1365) to the centrifuge tube and gently pipette up and down 2-3 times to resuspend the cells.
5. Carefully pour the cell suspension into two wells of a 6-well plate (0.7 to 1.5 million cells per well) or into a 100 mm culture dish.
6. After approximately 70%-80% of the cells have adhered (2-8 hours), gently aspirate the supernatant (to remove cell debris and non-adherent cells) and replace with pre-warmed maintenance medium (Cat. No. M1365, Cell Biologics).
7. Cells should be checked daily under a microscope to verify appropriate cell morphology.
8. Proceed with experiment assays within 1-3 days (when cells have reached 90-100% confluence) after plating cells.

Note: The numbers of seeding cells in each well may need to be modified according to the user's experience.  
Recommended seeding density: 200,000 viable cells/cm<sup>2</sup>.