

## Instruction Manual: Primary Hepatocyte culture with ReproHP Medium™

Cat. No. RCRM201 (and RCRM201-S)

Version 1.0

### Overview

This protocol describes proper procedures for the cell culture of primary human hepatocyte in ReproHP Medium™.

To maintain sterility, all cultivation and handling procedures should be performed inside a biological safety cabinet.

### Condition of use

**This product is for research use only. It should not be used for therapeutic or diagnostic purposes. Sale of this product to a third party, or any commercial use for the product, is prohibited without prior permission from ReproCELL.**

### Storage

ReproHP Medium™ should be stored at -20°C upon receipt. After thawing and reconstitution of the complete medium, store at 2-8°C and use within three weeks. Avoid repeated freezing and thawing.

### Characterization of ReproHP Medium™

- Each lot is tested for osmolality, pH, sterility, and mycoplasma.
- ReproHP Medium™ is provided serum-free, but requires the addition of Fetal Bovine Serum (FBS) for reconstituting the complete medium.

### General notes

- Complete ReproHP Medium™ consists of ReproHP™ Basal Medium, ReproHP™ Supplement (1.62µL/mL), and FBS (5%).
- Equilibrate ReproHP Basal Medium and ReproHP Supplement to room temperature before use.
- Minimize the exposure of ReproHP Supplement and complete **ReproHP Medium™** to bright light to avoid accelerated decomposition.

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## ReproHP Medium™

Description	Cat. No.	Format	Storage
ReproHP Basal Medium	N/A	500 ml	-20 °C
ReproHP Supplement	N/A	162 µl x 5 vials	-20 °C

## Required Reagents and Equipment

Description	Cat. No.	Format	Storage
Collagen coated plates	Various suppliers	Multi-well	
Primary Human Hepatocytes	Various suppliers		Usually liquid N <sub>2</sub>
FBS (Fetal bovine serum)	Various suppliers		-20°C
Cell Culture incubator (5% CO <sub>2</sub> )	Various suppliers		
Other Reagents as needed	Various suppliers	-	-

## ReproHP Medium™ Protocols

### Material preparation

#### Preparation of Complete ReproHP Medium

1. Thaw ReproHP Basal Medium at 4°C (usually takes overnight to one day).
2. Equilibrate required amount of ReproHP Basal Medium and ReproHP Supplement to room temperature prior to use.

**Note :** One tube of the supplement is enough to prepare 100 ml of complete ReproHP Medium. It is recommended to prepare the medium in multiples of 100 ml to avoid refreezing of components.

3. Add 162µl (one tube) of ReproHP Supplement to 95 ml ReproHP basal medium; mix well.
4. Also add 5 ml FBS to the basal medium (~90 ml) at a final concentration of 5% and mix well. The total volume of complete medium will be ~100 ml.

## Cultivating Primary Hepatocytes in ReproHP Medium

### Thawing and Seeding of Primary Hepatocytes in complete ReproHP Medium

**Note:** One conical tube of complete ReproHP Medium should be used to thaw / revive one vial of frozen hepatocyte cells. The minimum amount of medium needed for cell revival is dependent on the cell preparation and varies from ~20-50 ml (variation among cell suppliers). Our protocol is written for 50 ml of medium, which works well for all.

1. Aliquot 49 ml of complete ReproHP Medium into a 50 ml tube and equilibrate at 37°C in a water bath.
2. Remove the cryogenic vial containing the primary hepatocytes from liquid nitrogen storage, and thaw in a 37°C water bath (or according to the supplier's recommendations if indicated otherwise).
3. Withdraw the cell suspension by using a P1000 Pipetman and add the cells to the pre-warmed (complete) ReproHP Medium (49 ml).
4. Withdraw 1 ml (from the same 50 ml tube of cells) to rinse the residual cells from the cryogenic vial. Add the 1 ml rinse back into the same 50 ml tube.
5. Centrifuge the 50 ml tube of primary hepatocytes at 100xg for 10 minutes at room temperature.
6. Remove the supernatant and suspend the cells in complete ReproHP Medium. Count the cells and determine the cell density *by your usual method (hemocytometer, cell counter, etc)*. The resuspension volume you decide depends on the number of cells you require for your experiment size and plate choice.

**Note :** One hepatocyte cell vial is usually packaged to accommodate one 96 well plate. The total volume required for a 96 well plate is 9.6 ml (96 x 0.1 ml/well). Therefore, a typical resuspension volume for step 6 (above) is around 2-5 ml.

7. Once you have counted and determined your cell density (cells per ml), dilute the suspension in complete ReproHP Medium such that the required number of cells per well (see cell supplier recommendation) is contained in the volumes listed below. This varies depending on plate size or well number.

Table 1. Recommended cell culture medium volumes (per well) for multi-well plates

size	96 well plate	24 well plate	12 well plate	6 well plate
cm <sup>2</sup>	3.8	9.6	21.3	59
volume	100 µl	500 µl	1 ml	2 ml

- Add the cell suspension to each well of a collagen 1 -coated plate and incubate at 37°C in a 5% CO<sub>2</sub> incubator for 4 hours (*this allows time for cell attachment*).

**Note :** Most protocols recommend collagen 1-coated plates for hepatocyte adherence. However, follow the recommendation of the cell supplier if indicated otherwise.

- After 4 hours, remove the supernatant carefully, and then replace medium with fresh complete ReproHP Medium in each well according to volumes in Table 1.

**Note :** Avoid drying of cells during medium change.  
**Note :** Gently add medium to avoid cell detachment.

- Incubate the plate at 37°C in a 5% CO<sub>2</sub> incubator.

### Maintenance of Adherent Cells

Change the medium four hours after seeding and **every day** thereafter according to the recommendations below.

- Pipet the appropriate amount of complete ReproHP Medium to a sterile container. Warm the medium to 37°C in a water bath. Return the stock (unused) complete ReproHP Medium to the 4°C refrigerator and save for the next day.

**Note :** Complete ReproHP Medium is stable for at least 3 weeks when stored at 2-8°C (avoid light).

- Retrieve the plate of cells from the 37°C incubator and remove the medium. Immediately replace it with the warmed, fresh complete ReproHP Medium and return the plate to the incubator.

**Note :** For drug exposure, add the desired drug / chemical compound into the complete ReproHP Medium prior to the medium change.

## Frequently Asked Questions

### Q1. Can complete ReproHP Medium be refrozen for future use?

A1. It is possible to refreeze the complete ReproHP Medium one time only. Thawing should be carefully performed at 2-8°C.

### Q2. Can ReproHP Medium be used without addition of 5% FBS ?

A2. For cell revival from liquid nitrogen storage, 5% FBS is absolutely required. However, for cell maintenance and during assay conditions, FBS is currently recommended. Without FBS, the high performance of ReproHP Medium may be affected, with a partial decline in CYP expression or enzyme activity.

### Q3. Can ReproHP Medium be used with immortalized cell lines such as HepG2 or HepRG ?

A3. Only minimal data exists for CYP expression on HepG2 or HepRG cell lines. Cell maintenance is robust, with CYP basal expression and induction at least equivalent to other leading mediums.

### Q4. Is ReproHP Medium available without Phenol Red?

A4. A formulation of ReproHP without Phenol Red is currently not available, but under development.

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