

I. Introduction

A. Summary

This Protocol-At-A-Glance is used for extended maintenance of human pluripotent stem cell-derived hepatocytes generated by the Cellartis iPS Cell to Hepatocyte Differentiation System (Cat. No. Y30055) or the Cellartis Hepatocyte Differentiation Kit (Cat. No. Y30050). Provided as a volume of 100 ml, the Cellartis Hepatocyte Maintenance Medium will extend the experimental time window of the hepatocytes for an additional 10 days.

B. General Considerations

Storage & Handling

- Store Cellartis Hepatocyte Maintenance Medium at -20°C ; shelf life is indicated on the product label.
- Thaw Hepatocyte Maintenance Medium at RT; use thawed Hepatocyte Maintenance Medium within three days (or aliquot and freeze once it has been thawed).

NOTES:

- 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 during media changes, leave a small volume (about 10% of the total volume) of liquid in each well prior to adding fresh medium.

II. Protocol: Medium Change of Hepatocytes

Use this protocol to change the medium on hepatocytes derived using the Cellartis iPS Cell to Hepatocyte Differentiation System or the Cellartis Hepatocyte Differentiation Kit. Schedule media changes for Monday, Wednesday, and Friday to avoid media changes over the weekend.

NOTE: Always work under aseptic conditions.

A. Preparing the Medium

Thaw Hepatocyte Maintenance Medium at RT. Once thawed, Hepatocyte Maintenance Medium can be stored at $2-8^{\circ}\text{C}$ for up to three days. One bottle of Hepatocyte Maintenance Medium provides enough reagent for two media changes for hepatocytes grown on 50 cm^2 of culture surface.

B. Changing the Medium

1. 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/cm^2 of warm Hepatocyte Maturation Medium.

NOTE: Do not allow the cells to dry out during media changes.

2. Incubate the cells at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 5% CO_2 , and $\geq 90\%$ humidity until the next medium change or until hepatocytes are to be used in experiments.

NOTE: To image hepatocytes during a medium change, take images within 1–2 minutes after the medium has been removed, and then immediately replace with fresh medium.

3. Discard any unused warmed medium.

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