# Activated Myofibroblastic Hepatic Stellate Cells

**Catalog Numbers** HMFHSC

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

**IMPORTANT!** Cryopreserved stellates should be kept at all times in the vapor phase of liquid nitrogen. The product shall only be removed when it is needed and thawed immediately after removal from the liquid nitrogen container. Cryopreserved hepatic stellates must be stored at all times in the vapor phase of liquid nitrogen, not submerged. The product should only be removed when it is to be thawed and cultured, which should occur immediately after removal from liquid nitrogen storage.

## Product description

Activated Myofibroblastic Hepatic Stellate Cells (MF-HSCs) are produced from isolated pure quiescent human stellate cells (q-HSCs). The q-HSCs are activated to become MF-HSCs through a process of passaging and maintence in cell culture media. These activated MF-HSCs are ideal for building hepatic co-culture with primary hepatocytes to study in vitro disease models such as hepatic fibrosis. The cells are obtained from a single donor and provided in a cryopreserved format identical to single donor plateable hepatocytes. Additionally, every lot of cells is tested for cell viability, plating efficiency, morphology, purity based on $\alpha$-smooth muscle actin ($\alpha$-SMA) expression, and activation in response to Transforming Growth Factor-$\beta$1 (TGF-$\beta$1).

## Contents and storage

| Contents | Amount | Shipping | Storage |
|---|---|---|---|
| Activated Myofibroblastic Hepatic Stellate Cells | 1 mL (>1.0 × $10^6$ cells) | Liquid nitrogen (vapor phase) | Liquid nitrogen (vapor phase). |

## Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

| Item | Source |
|---|---|
| Nunc™ 15 mL & 50 mL Conical Sterile Polypropylene Centrifuge Tubes | 339651, 339653 |
| RNase-Free Microfuge Tubes | AM12400 |
| Collagen I, Coated Plate, 6- or 24-well | A1142801, A1142802 |
| DPBS, no calcium, no magnesium | 14190144 |
| StemPro™ Accutase™ Cell Dissociation Reagent | A1110501 |
| Trypan Blue Solution, 0.4% | 15250061 |
| Advanced DMEM | 12491015 |
| GlutaMAX™ Supplement | 35050061 |
| Fetal Bovine Serum, certified, US origin | 16000044 |
| Penicillin-Streptomycin (10,000 U/mL) | 15140122 |

## Product specifications

| Description | Details |
|---|---|
| Form | Cryopreserved |
| Species | Human |
| Quantity | 1 CryoVial |
| Donor source | 1 donor |
| Product size | 1 mL |
| Number of cells | 1 × $10^6$ cells |

## Procedural guidelines

- Cryopreserved cells should be thawed quickly in a 37°C water bath.
- Once thawed, cryopreserved MF-HSC must be used immediately and cannot be refrozen.

## Prepare activated/myofibroblastic stellate cell plating media (MF-Plating Media)

Aseptically mix the following media and reagents:

| Reagent | Amount |
|---|---|
| Advanced DMEM | 500 mL |
| GlutaMAX™ Supplement | 5 mL |
| Fetal Bovine Serum, certified, US origin (final concentration ~10%) | 50 mL |
| Penicillin-Streptomycin (1X final concentration) | 5 mL |

MF-Plating Media can be saved in 4°C, protected from light, up to one month.

## Thaw and plate cells

1. Warm up the plating media prepared above in a 37°C water bath for at least 30 minutes before thawing cryopreserved cells.
2. Decontaminate the external surfaces of the stellate plating media bottle with 70% ethanol and transfer it to a biosafety cabinet.
3. In a 15-mL centrifuge tube, add 9 mL of activated myofibroblastic stellate cell plating media from Step 1.
4. Remove the vial of cells to be thawed from liquid nitrogen and rapidly thaw by placing in a 37°C water bath with gentle agitation for 1–2 minutes (or once a sliver of ice is left in the tube).

   Complete thawing can be detrimental to the cell viability.
5. Decontaminate the external surface of the cryovial with 70% ethanol. Transfer the vial into the biosafety cabinet and promptly transfer the contents of the vial to a 15-mL centrifuge tube containing 9 mL of plating media.
6. Centrifuge the 15-mL centrifuge tube containing the stellate cells at 500 × *g* for 5 minutes at room temperature.
7. Carefully aspirate the supernatant.

   Make sure not to disturb the pelleted stellate cells.
8. Resuspend the cells in 1 mL of pre-warmed stellate cell plating media by gently pipetting up and down.
9. Take 10–20 µL of the resuspended cells and count them using hemocytometer or any other counting device.
10. Plate the cells in collagen I coated plates by diluting to approximately 10,000 cells/well for a 24-well plate or 40,000 cells/well for a 6-well plate to achieve a 30–40% confluency after 24-hours.

    We recommend that you seed the cells in 500 µL plating media per well for 24-well plates and 2-mL plating media per well for 6-well plates.
11. Place the culture plate in a 5% $CO_2$ incubator maintained at 37°C. In a back-and-forth and side-to-side motion, shake the plate to evenly and gently distribute the cells in the culture well.
12. Culture the cells undisturbed for at least 12–16 hours before using them for experimentation.

## Change media

When culturing MF-HSCs longer than three days, the media should be changed once every three days.

1. Warm the plating media and DPBS in a 37°C water bath.
2. In the biosafety cabinet, aspirate the media from the culture plate. Rinse the stellate cells with pre-warmed DPBS with 1 mL per well for the 24-well plate or 4 mL per well for the 6-well plate.
3. Aspirate DPBS away from the plated cells.

   Make sure not to disturb the plated cells.
4. Add 0.5 mL of plating media per well for the 24-well plate or 2 mL of plating media per well for the 6-well.
5. Return the culture plate to the 5% $CO_2$ incubator maintained at 37°C.

**IMPORTANT!** We do not recommend further passaging of hepatic stellate cells, because further passaging will reduce the level of activation of the HSCs.

## Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

| Item | Source |
|---|---|
| Human Plateable Hepatocytes, Induction Qualified | HMCPIS |
| Human Plateable Hepatocytes, Transport Qualified | HMCPTS |
| Spheroid Qualified Human Hepatocytes | HMCPSQ |

## Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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