

Hepatocyte differentiation protocol

A protocol for the differentiation of embryonic stem cells and induced pluripotent cells into hepatocytes.

Protocol kindly provided by Chad Cowan's group, Harvard Stem Cell Institute.

Preparation of media

All media should be carried out in a tissue culture hood under aseptic conditions. Media should be freshly made and not used if stored for more than two weeks.

Basal medium

RPMI-1640 + B27 supplement + penicillin/streptomycin (100 U/ml penicillin, 100 µg/ml streptomycin, final concentration).

Use basal medium to make definitive endoderm (DE), hepatic endoderm (HE) and immature hepatocyte (IMH) media.

DE medium

Basal medium
100 ng/ml Activin A
3 µM CHIR99021

HE medium

Basal medium
5 ng/ml basic fibroblast growth factor (bFGF)
20 ng/ml bone morphogenic protein 4 (BMP4)
0.5% DMSO

IMH medium

Basal medium
20 ng/ml hepatocyte growth factor (HGF)
0.5% DMSO

Mature hepatocyte (MH) medium

Hepatocyte basal medium + singleQuots®
20 ng/ml HGF
20 ng/ml Oncostatin M
100 nM dexamthasone
0.5% DMSO

Hepatocyte differentiation

1. Coat plates with Matrigel.
2. **Day 1:** Split ES/iPS cells and plate at a density of 1.4×10^5 cells/ml in mTES with 4 µM of the ROCK inhibitor Y27632. Plate 1 ml per well. Plating density may have to be optimized for each different cell line.

3. **Day 2:** Start treating cells with DE medium. Remove mTESR and replace with 1 ml DE medium per well of the six-well plates.
4. **Day 3-4:** Change DE medium daily. Cells will become spiky and ES cells should disappear or die off. At the end of this stage, you will have definitive endoderm cells (Figure 1).

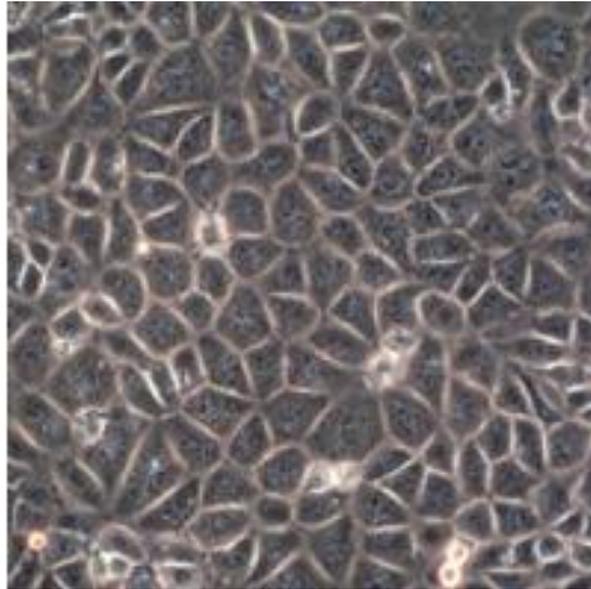


Figure 1. Definitive endoderm cells at Day 4

5. **Day 5:** Start treating cells with HE medium for 5 days. Replace DE medium with 1 ml HE medium per well.
6. **Day 6–9:** Change HE medium daily. At the end of this stage, you will have hepatic endoderm cells (Figure 2).

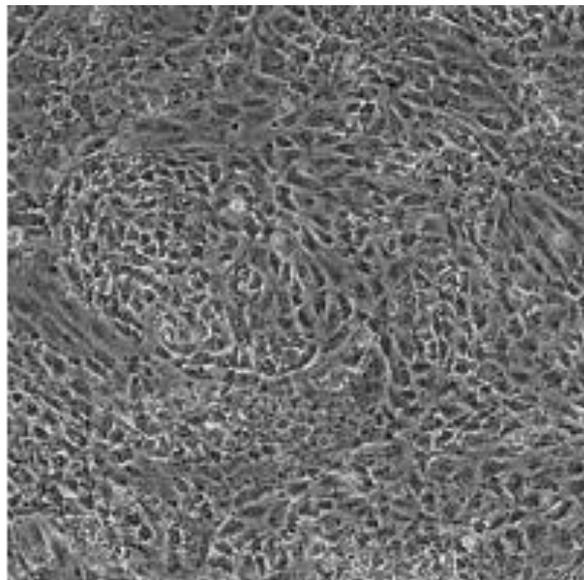


Figure 2. Hepatic endoderm cells after treatment with HE medium

7. **Day 10:** Start treating cells with IMH for 5 days. Replace HE medium with 1 ml IMH medium per well.
8. **Day 11–14:** Change IMH medium daily. At the end of this stage you will have immature hepatocyte-like cells (Figure 3).

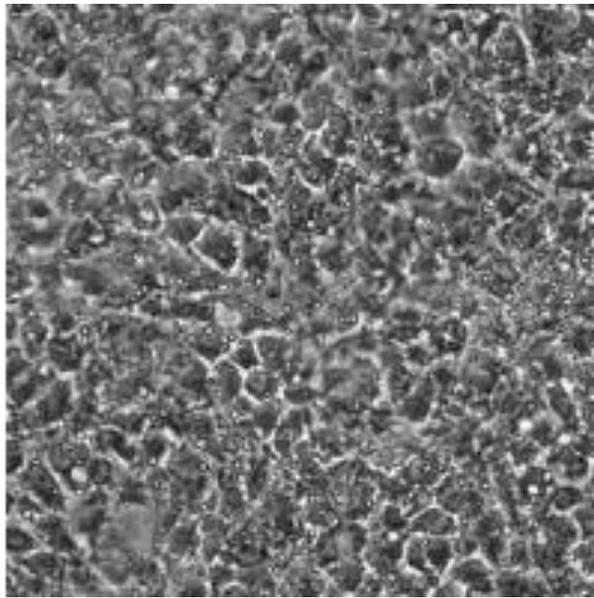


Figure 3. Immature hepatocyte-like cells at Day 15

9. **Day 15:** Start treating cells with MH medium for 10–12 days. Replace IMH medium with 1 ml MH medium per well.
10. **Day 16–24:** Change MH medium daily. At the end of this stage you will have mature hepatocyte-like cells (Figure 4).

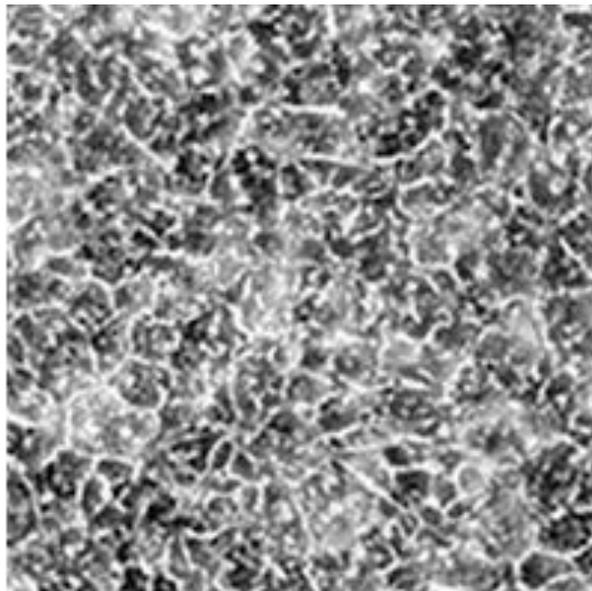


Fig 4. Mature hepatocyte-like cells at Day 20

Cells can be kept up to 30 days in total, keeping cells for longer is not recommended.