

Product No. : 20607 / 20612 / 20683 / 20615

Hepatocyte Differentiation Medium

Hepatocyte Maintenance Medium

AscleStem® Hepatocyte Differentiation Medium and AscleStem® Hepatocyte Maintenance Medium is a serum free cell culture medium to differentiate hPSCs to hepatocyte or cultivate hepatocyte. For a differentiation, AscleStem® Hepatocyte Differentiation Basal Medium (#20607-05) should be mixed with Supplement for H.D. Medium (#20612-54) and for a maintenance, AscleStem® Hepatocyte Maintenance Basal Medium (#20683-05) should be mixed with Supplement for H.M. Medium (#20615-24). (adding cytokines is required)

Please read beforehand

- To thaw Supplement for H.D. Medium and Supplement for H.M. Medium, put them in a refrigerator and thaw them gently, but not put them in a 37°C-temperated water bath.
- Filter sterilization will not be necessary when mixing step has been done under sterile environment.
- Store a mixed medium at a refrigerator protecting from light.
- Take immediately the mixed medium back to the refrigerator after use to avoid getting degradation from letting it stay for a long period at room temperature.
- For a cultivation, aliquot the mixed medium to a smaller container, add necessary cytokines, and warm it at 37C in a water bath.

Other requirements

Cytokines (Human/Mouse/Rat, Activin A, Human BMP4, Human FGF4, Human HGF, Human Oncostatin M)

Preparation

Hepatocyte differentiation medium

- (1) Thaw Supplement for H.D. Medium completely
- (2) Mix them as following:

AscleStem® Hepatocyte differentiation medium	500 mL
Supplement for H.D. Medium	5 mL

The mixed medium refers to "Hepatocyte differentiation medium" in the protocol below.

Hepatocyte maintenance medium

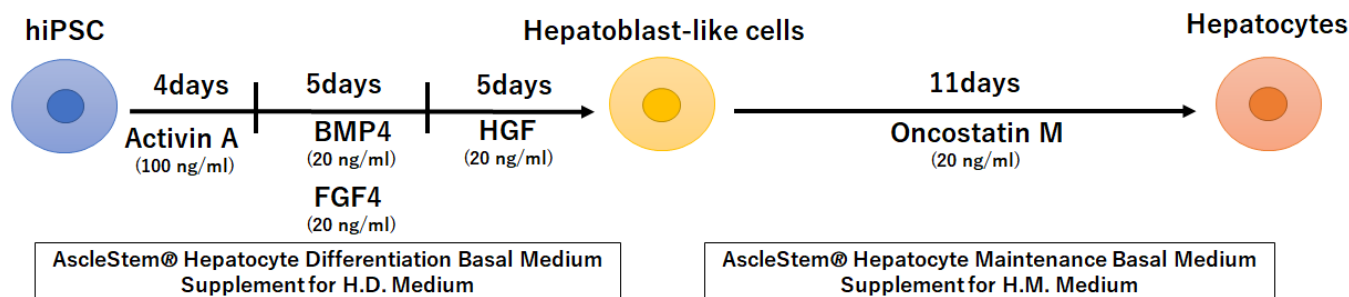
- (1) Thaw Supplement for H.M. Medium completely
- (2) Mix them as following:

AscleStem® Hepatocyte maintenance medium	500 mL
Supplement for H.M. Medium	30 mL

The mixed medium refers to "Hepatocyte maintenance medium" in the protocol below.

Protocol

Protocol for differentiation with 12-well plate



[Coating]

- (1) Coat required cell culture apparatuses with a coating agent^{※1}.
- (2) Incubate at 37°C, CO₂ 5%, until use.

※1 【Matrigel®】 is recommended as a coating agent

[hiPSCs cultivation]

•With feeder cell conditions

- (1) Cultivate hiPSCs in a 100 mm dish until 60 - 70% confluent^{※2}.
Dissociate cells by a enzymatic cell dissociation reagent^{※3}.
- (2) Centrifuge a vial with 160×g for 5 minutes, add an appropriate volume of maintenance medium, and break down to 300 - 200 cells/clump by pipetting.
- (3) Cultivate the cells evenly added to a each well of a coated 12-well plate (suitable cell density depends on a type of cell line) at 37°C in 5% CO₂ incubator.

※2 Cell numbers of 60% confluent in 100 mm dish is enough for those of a 12-well plate.

※3 【AscleStem® Dissociation Solution for hPSCs】 or 【1 mg/ml Dispase】 is recommended.

•Feeder-free conditions

- (1) Cultivate hiPSCs in a 100 mm dish until 60 - 70% confluent.
- (2) Dissociate cells by enzymatic cell dissociation solution^{※4}.
- (3) Centrifuge a vial with 300×g for 5 minutes, suspend the cells with maintenance medium for iPSCs containing Y-27632, and count the cell numbers.
- (4) Cultivate the cells in a coated cell culture plate at 60,000 - 120,000 cells/cm² (suitable cell density depends on a type of cell line) at 37°C in 5% CO₂ incubator.
- (5) Cultivate the cell until being 80% confluent. Changing maintenance medium for iPSCs may be necessary.

※4 【Accumax™】 is recommended.

[Schematic cell culture step]

(Day0, 1, 2, 3)

- (1) Confirm 80% confluent.
- (2) Add activin A to “Hepatocyte differentiation medium”, then change the medium every 24 hours.

(Day4, 5, 6, 7, 8)

- (3) Add BMP4 and FGF4 to “Hepatocyte differentiation medium”, then change the medium every 24 hours.

(Day9, 10, 11, 12, 13)

- (4) Add HGF to “Hepatocyte differentiation medium”, then change the medium every day.

(After Day 14, every other day)

- (5) Add Oncostatin M to “Hepatocyte maintenance medium”, then change the medium every other day.

[Required reagents]

Product No.	Manufacturer	Product Name
18585-36	Nacalai Tesque	Activin A Solution, Human, Recombinant
17087-54	Nacalai Tesque	Accumax™
18190-96	Nacalai Tesque	10mmol/L-Y-27632 Solution
21777-84	Nacalai Tesque	AscleStem® Dissociation Solution for hPSCs
AF-120-05ET	Pepro Tech	Animal-Free Recombinant Human BMP-4 (E.coli derived)
AF100-31	Pepro Tech	Animal-Free Recombinant Human FGF-4
04267-94	Pepro Tech	HGF, Human Recombinant (Insect derived)
AF-300-10T	Pepro Tech	Animal-Free Recombinant Human Oncostatin M (209 a.a.)
354230	Corning	Matrigel® Matrix Basement Membrane Growth Factor Reduced

Precaution

(Hepatocyte differentiation medium)

- Since dead cells float a lot during 3-day cultivation after induction of differentiation, changing medium at every 24 hours is recommended.
- Dead cells are not appeared after day 7.
- Since hepatocyte differentiation medium needs added 4 kinds of cytokines, aliquoting medium when using is recommended.
- Need to pretest in case that an appropriate concentration of cytokines maybe differs to iPS cell lines.
- When adding other cytokines, rinsing cells at medium change is not necessary.
- Storage prepared medium in a refrigerator protecting from light and use up within 2 weeks.

(Hepatocyte maintenance medium)

- Although AscleStem® Hepatocyte Maintenance Basal Medium changes color to light yellow when mixed with Supplement for H.M. Medium, it is no problem on its quality.
- Storage prepared medium in a refrigerator protecting from light and use up within 2 weeks.
- By adding hEGF, this product is applicable for primary human hepatocyte, too.

Storage

Product	Storage
AscleStem® Hepatocyte Differentiation Basal Medium	2 - 8°C
Supplement for H.D. Medium	-20°C
AscleStem® Hepatocyte Maintenance Basal Medium	2 - 8°C
Supplement for H.M. Medium	-20°C

Expiry date

Indicated on a label.

Component

Product No.	Product	Size
20607-05	AscleStem® Hepatocyte Differentiation Basal Medium	500 mL
20612-54	Supplement for H.D. Medium	5 mL
20683-05	AscleStem® Hepatocyte Maintenance Basal Medium	500 mL
20615-24	Supplement for H.M. Medium	30 mL

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