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User Manual

OriCell™ Human Mesenchymal Stem Cell Hepatogenic Differentiation Medium

Cat. No. HUXMX-90101

PRODUCT DESCRIPTION:

In vitro models of parenchymal liver cells are of great scientific value in toxicology and bioartificial liver research. However, the primary cell cultures of hepatocytes are hindered by their short lifespan and rapid loss of hepatic function under *in vitro* conditions.

Human mesenchymal stem cells (hMSCs) possess a versatile differentiation potential ranging from mesenchymal-related multipotency to ectodermal and endodermal competency. Of particular interest, is their hepatogenic potential which can be used for liver-directed stem cell therapy and transplantation.

The OriCell™ Human Mesenchymal Stem Cell Hepatogenic Differentiation kit provides an optimal system designed for the hepatogenic differentiation of human mesenchymal stem cells. This kit consists of a Pretreatment Medium, a Hepatocyte Differentiation Medium, and a Hepatocyte Maturation Medium. All components are supplied ready-to-use for hepatocyte differentiation.

This product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

KIT COMPONENTS:

Pretreatment Medium

Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium A (Cat. No. HUXMX-03101-50)	50 mL
EGF	10 µL
bFGF	5 µL

Hepatocyte Differentiation Medium

Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium B (Cat. No. HUXMX-03101-100)	100 mL
HGF	20 µL
bFGF	10 µL
Nicotinamide	100 µL

Hepatocyte Maturation Medium

Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium C (Cat. No. HUXMX-03101-200)	200 mL
Oncostatin M	40 µL
Dexamethasone	100 µL
ITS+Premix	2 mL

INSTRUCTIONS:

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient 0.1% Gelatin Solution into the appropriate culture vessel to completely cover its base.
2. Swirl the vessel until Gelatin Solution coats entire base of vessel. Let sit for at least 30 minutes at room temperature.
3. Aspirate off all of the Gelatin Solution and allow the remainder to evaporate by leaving the vessel sitting open in the hood for no more than 30 minutes.
4. Enclose the culture vessel once it has dried and store at 4℃ until use.

Hepatogenesis Protocol (for 6-well tissue culture plate):

1. Human Mesenchymal Stem Cells are cultured in Human Mesenchymal Stem Cell Growth Medium (Cat. No. HUXMX-90011) (growth medium thereafter) at 37°C in a 5% CO_2 humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with Trypsin-EDTA (Cat. No. TEDTA-10001).
3. Human Mesenchymal Stem Cells are replated in growth medium at a density of 1.5×10^4 cells/cm^2 in a 6-well tissue culture plates that has been pre-coated with 0.1% Gelatin Solution.
4. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

Pretreatment Stage (Duration of 2 days)

1. After 24 hours, prepare Pretreatment Medium and begin the Pretreatment Stage. To prepare the Pretreatment Medium, thaw EGF and bFGF solution at room temperature. Gently invert the vials several times to ensure homogeneity.

Note: **Centrifuge the vials briefly at low speed before removing the caps to ensure recovery of their entire content.**

2. Transfer the entire amount of EGF and bFGF into Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium A. Gently swirl the fully supplemented complete medium to ensure a homogeneous mixture. Pretreament Medium is now ready to use.
3. Carefully aspirate off the growth medium from each well and add 2 mL of the Pretreatment Medium to each well.
4. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

Hepatocyte Differentiation Stage (Duration of 7 days)

1. After two days of pretreatment, prepare Hepatocyte Differentiation Medium and begin the Hepatocyte Differentiation Stage.

Note: **Hepatogenic Differentiation Medium should be made fresh for each cycle. It should be store at 4℃ for up to 2-7 days.**

2. Mix the following sterile components with 10 mL Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium to make 10 mL of complete Hepatocyte Differentiation Medium. Scale up or down according to experimental design.

Component	Amout of stock solution
HGF	2 µL
bFGF	1 µL
Nicotinamide	10 µL

3. Change the medium to Hepatogenic Differentiation Medium by completely replacing the spent pretreatment medium.
4. Refeed cells every 3 days for a total of 7 days by completely replace the medium with fresh Hepatogenic Differentiation Medium.

Hepatocyte Maturation Stage (7-14 days)

1. After 7 days of differentiation, prepare Hepatocyte Maturation Medium, and begin the Hepatocyte Maturation Stage.

Note: **Hepatocyte Maturation Medium should be made fresh for each cycle. It can be stored at 4℃ up to 7 days.**

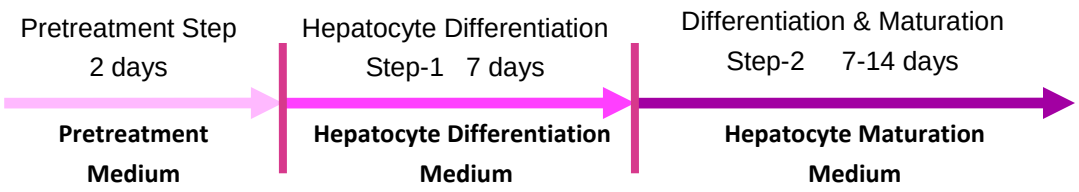
2. Mix the following sterile components with 10 mL Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium to make 10 mL of complete Hepatocyte Maturation Medium. Scale up or down according to experimental design.

Component	Amout of stock solution
Oncostatin M	2 µL
Dexamethasone	5 µL
ITS+Premix	100 µL

3. Change the medium to Hepatogenic Muturation Medium by completely replacing the spent hepatocyte differentiation medium.
4. Refeed cells every 3 days for 7 to 14 days by completely replace the medium with spent Hepatogenic Muturation Medium.
5. After 7 to 14 days of maturation, cells can be collected for various evaluation tests.

Hints:

1. Overview: 16-23 days protocol for human MSCs hepatogenic differentiation



2. Use extreme care when changing the medium as the cells are adhere loosely in the absence of serum.
3. Cells before differentiation exhibit a fibroblast-like morphology, and do not change significantly during the differentiation step-1. About 12 days differentiation, cells develop a broadened flattened shape. During the maturation step, a polygonal cell shape develops.

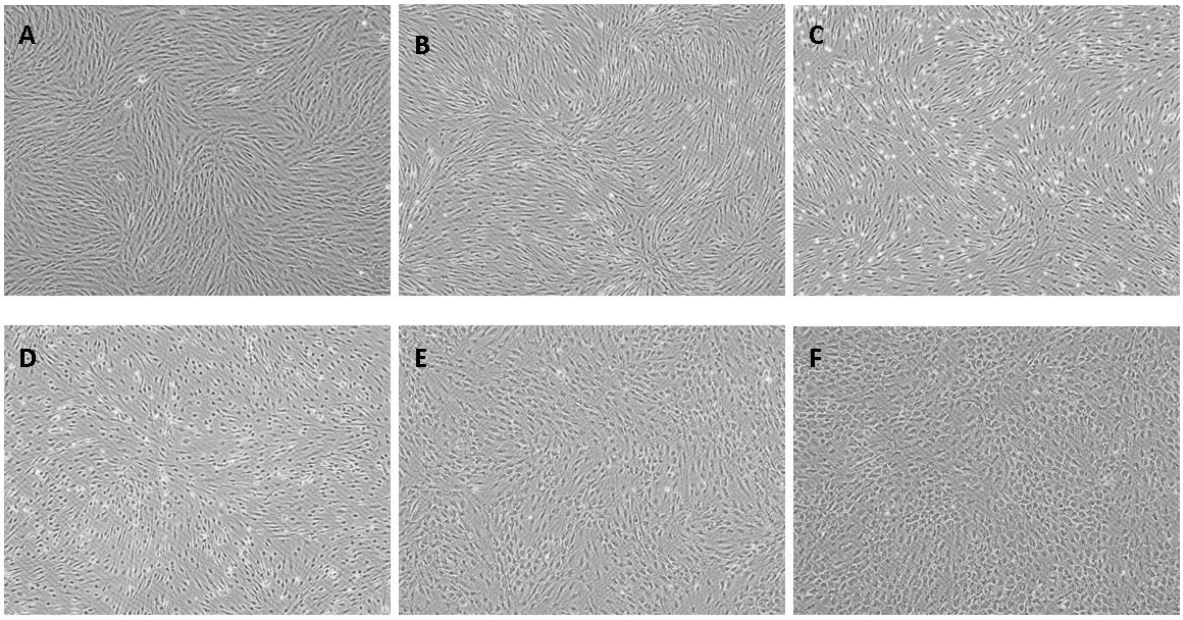


Fig. 1 Morphology of human mesenchymal stem cells during the differentiation protocol.

Undifferentiated MSCs (**A**); 3 days postinduction (**B**); 6 days postinduction (**C**); 9 days postinduction (**D**); 12 days postinduction (**E**); 16 days postinduction (**F**). The morphology of matured hepatocyte-like cells is achieved at 12 days postinduction and retained for two weeks. (magnification x 100)

4. Differentiated hepatocyte mature cells should maintain longer if re-seeded in tissue culture plates that have been pre-coated with laminin or matrigal before induction.
5. It is strongly recommended to use MSCs with less than 8 passages. MSCs will gradually lose their multipotency with increasing passage number.

STABILITY AND STORAGE:

All products should be stored in the dark.

Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium is stable at 2 to 8°C for up to one year. Other components are stable at -20°C for 3 months to a year. These products should be discarded beyond the labeled expiration date.

Once prepared, the fully supplemented complete medium can be stored for up to one week when stored in the dark at 2 to 8°C.

For optimal performance, repeated warm-cooling and freeze-thawing should be avoided. It is recommended that medium supplements are aliquoted according to the experimental design in order to prevent excessive freeze-thaw cycles.

QUALITY CONTROL:

Human Mesenchymal Stem Cell hepatogenic Differentiation Medium is performance tested on Human Mesenchymal Stem Cells.

Standard evaluation includes:

1. Sterility test (bacteria, fungi and mycoplasma)
2. pH test
3. Osmolality
4. Endotoxin

TECHNICAL SUPPORT:

Please visit the Cyagen website at www.cyagen.com for technical resources, additional product information, and special offers. You may also write, email, call, or fax to us at:

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Material Safety Data Sheets (MSDSs) are available upon request.

The Certificate of Analysis (CoA), which provides detailed quality control information for each product, is also available at the Cyagen website.