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User Manual

OriCell™ Mesenchymal Stem Cell Chondrogenic Differentiation Medium

Cat. No. GUXMX-90041

PRODUCT DESCRIPTION:

OriCell™ Mesenchymal Stem Cell Chondrogenic Differentiation Medium consists of optimized Mesenchymal Stem Cell (MSC) Chondrogenic Differentiation Basal Medium and cell culture supplements. This product has been developed for the optimal differentiation of mesenchymal stem cells (MSCs) into cartilages.

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

KIT COMPONENTS:

Mesenchymal Stem Cell (MSC) Chondrogenic Differentiation Basal Medium (Cat. No. GUXMX-03041)	194 mL
Dexamethasone	20 µL
Ascorbate	600 µL
ITS+Supplement	2 mL
Sodium Pyruvate	200 µL
Proline	200 µL
TGF-β3	2 mL
Alcian Blue	10 mL

INSTRUCTIONS:

Preparation of the Incomplete Chondrogenic Induction Medium

1. Disinfect the external surfaces of the bottles/vials for the following components in the kit with 70% v/v ethanol. Allow ethanol to evaporate.
   a) Dexamethasone
   b) Ascorbate
   c) ITS+Supplement
   d) Sodium Pyruvate
   e) Proline
   f) MSC Chondrogenic Differentiation Basal Medium
2. Aseptically open the above components inside a laminar flow hood. Add the contents into the MSC Chondrogenic Differentiation Basal Medium to prepare the Incomplete Chondrogenic Induction Medium.
3. Rinse each vial with the medium. Although it may not be possible to recover all of the content of each component after rinse, small losses should not affect cell characteristics.
4. The Incomplete Chondrogenic Induction Medium is now ready to use. Store at 2-8°C in the dark until needed.

Aliquot TGF-β3

1. Aliquot small volume of TGF-β3 into freezer-safe tubes and store at –20°C or below for no more than 6 months.

***Important*: 10 µL of TGF-β3 will convert 1 mL of Incomplete Chondrogenic Medium into Complete Medium.**

2. Thaw the required amount of TGF-β3 needed in order to convert the incomplete induction medium into complete medium. You may need to centrifuge briefly at low speed in order to pull the small volume of TGF-β3 to the bottom of the tube.
3. Pipette in the volume of Incomplete Chondrogenic Induction Medium that
4. you intend to supplement into the tube containing TGF-β3.
5. Mix the solution by pipetting and then transfer it back to the tube of Chondrogenic Induction Medium. Repeat this process to be certain that you have recovered the TGF-β3. Cap and invert several times to mix.
6. The Chondrogenic Induction Medium is now complete.

***Important*: Complete Chondrogenic Medium must be prepared fresh and used within 12 hours.**

CHONDROGENESIS PROTOCOL:

1. Calculate the total number of MSC pellet cultures required for your experiment (2.5×10^5 MSCs are needed to form one chondrogenic pellet). Transfer this amount of cells into an appropriate culture tube.
2. Wash the MSCs with Incomplete Chondrogenic Medium. Centrifuge the cells at 150 x *g* for 5 minutes at room temperature and then aspirate off the supernatant. Resuspend the cells in 1 mL of Incomplete Chondrogenic Medium per 7.5×10^5 cells. Centrifuge again at 150 x *g* for 5 minutes and then aspirate off the medium.
3. Resuspend the MSCs in Complete Chondrogenic Induction Medium to a concentration of 5.0×10^5 cells/mL.
4. Aliquot 0.5 mL (2.5×10^5 cells) of the cell suspension into 15 mL polypropylene culture tubes. Centrifuge the cells at 150 x *g* for 5 minutes at room temperature. DO NOT aspirate the supernatant nor resuspend the pellet.
5. Loosen the caps of the tubes in order to allow gas exchange and incubate the tubes at 37°C in a humidified atmosphere of 5% CO_2. Do not disturb the pellets for 24 hours.
6. Feed the cell pellets every 2-3 days by completely replacing the medium in each tube (to avoid aspirating the pellets when aspirating the medium, attach a sterile 1-200µL pipette tip to the end of the aspirating pipette). Add 0.5 mL of freshly prepared Complete Chondrogenic Medium to each tube.
7. After replacing the medium, flick the bottom of the tube to ensure that the pellet is free floating. Loosen the caps and return the tubes to the 37°C incubator.
8. Chondrogenic pellets should be harvested after 14-28 days in culture. Pellets may be formalin-fixed and paraffin-embedded for alcian blue stain analysis.

ALCIAN BLUE STAINING PROCEDURE:

1. The tissue sample should be formalin-fixed and paraffin-embedded already.
2. Staining procedure:
   a) Deparaffinize slides and hydrate to distilled water.
   b) Stain in alcian blue solution for 30 minutes.
   c) Wash in running tap water for 2 minutes.
   d) Rinse in distilled water.
   e) Visualize under a light microscope and capture images for analysis. Blue staining indicates synthesis of proteoglycans by chondrocytes.

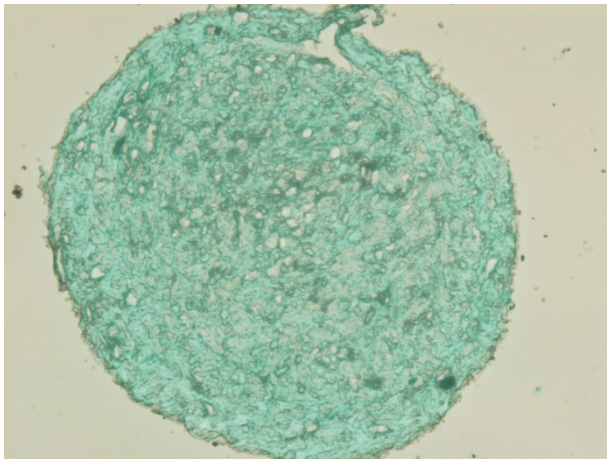

Fig.1 OriCell™ Human MSCs are differentiated into cartilages and are stained with alcian blue.

STABILITY AND STORAGE:

All products should be stored in the dark. Mesenchymal Stem Cell Chondrogenic Differentiation Basal Medium is stable at 2-8°C for up to one year. ITS+Supplement is stable for a minimum of 3 months at 2-8°C. Other components are stable at -20°C for up to two years.

These products should be discarded beyond the labeled expiration date. Once prepared, the fully supplemented (complete) medium can be stored for 12 hours when stored in the dark at 2-8°C.

For optimal performance, repeated warm-cooling and freeze-thawing should be avoided.

QUALITY CONTROL:

Oricell™ Mesenchymal Stem Cell Chondrogenic Differentiation Medium has been tested for performance on mesenchymal stem cells. The standard evaluation

includes:

- Sterility test (bacteria, fungi, and mycoplasma)
- pH test

- Osmolality
- Endotoxin

RELATED PRODUCTS:

Product	Catalog Number
OriCell™ Human Mesenchymal Stem Cells	HUXMA-01001
OriCell™ Wistar Rat Mesenchymal Stem Cells	RASMX-01001
OriCell™ F344 Rat Mesenchymal Stem Cells	RAFMX-01001
OriCell™ Rabbit Mesenchymal Stem Cells	RBXMX-01001
OriCell™ SD Rat Mesenchymal Stem Cells	RASMX-01001
OriCell™ Dog Mesenchymal Stem Cells	CAXMX-01001
OriCell™ Strain C57BL/6 Mouse Mesenchymal Stem Cells	MUBMX-01001
OriCell™ Strain BALB/c Mouse Mesenchymal Stem Cells	MUCMX-01001