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User Manual

OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation Medium

Cat. No. GUXMX-90031

PRODUCT DESCRIPTION:

OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation Medium consists of optimized Mesenchymal Stem Cell Adipogenic Differentiation Basal Media, cell culture supplements, and pre-selected fetal bovine serum. This product has been developed for the optimal differentiation of mesenchymal stem cells (MSCs) into adipocytes.

This 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) Adipogenic Differentiation Medium A:

Mesenchymal Stem Cell (MSC) Adipogenic Differentiation Basal Medium A (Cat. No. GUXMX-03031)	175 mL
Mesenchymal Stem Cell (MSC)-Qualified Fetal Bovine Serum (Cat. No. GUXMX-05001)	20 mL
Penicillin-Streptomycin	2 mL
Glutamine	2 mL
Insulin	400 µL
IBMX	200 µL
Rosiglitazone	200 µL
Dexamethasone	200 µL

Mesenchymal Stem Cell (MSC) Adipogenic Differentiation Medium B:

Mesenchymal Stem Cell (MSC) Adipogenic Differentiation Basal Medium B (Cat. No. GUXMX-03032)	175 mL
Mesenchymal Stem Cell (MSC)-Qualified Fetal Bovine Serum (Cat. No. GUXMX-05001)	20 mL
Penicillin-Streptomycin	2 mL
Glutamine	2 mL
Insulin	400 µL
Oil Red O	5 mL

INSTRUCTIONS:

Preparation of the MSC Adipogenic Differentiation Medium A (Induction Medium)

1. Prior to use, thaw the MSC-Qualified Fetal Bovine Serum at 2-8°C overnight or until completely thawed. Gently swirl the bottle to ensure homogeneity. The serum has been heat-inactivated and is ready to use after thawing.

***Note*: The thawed serum may contain some flocculent precipitates. The presence of these substances in serum does not alter the performance characteristics of the product. It is not recommended to filter the serum to remove these precipitates. Doing so may result in the loss of some serum nutrients.**

2. About 30 minutes prior to use, thaw Dexamethasone, Insulin, IBMX, Rosiglitazone, Penicillin-Streptomycin solution, and Glutamine 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 the entire content.**

3. Disinfect the external surfaces of the bottles/vials for every component in the kit with 70% v/v ethanol. Allow ethanol to evaporate.
4. Aseptically open the bottles/vials inside a laminar flow hood.
5. Transfer the entire amount of MSC-Qualified Fetal Bovine Serum, Penicillin-Streptomycin solution, and Glutamine solution into the MSC Adipogenic Differentiation Basal Medium A.
6. Rinse each vial/bottle with a little amount of basal medium A. Subsequently transfer the entire rinse medium back into the bottle of basal medium A.
7. Transfer the entire amount of Dexamethasone, Insulin, IBMX, and Rosiglitazone into the MSC Adipogenic Differentiation Basal Medium A. Rinse each vial with a small amount of basal medium A. Subsequently transfer the entire rinse medium back into the bottle of basal medium A.
8. Repeat step 7 several times.
9. Gently swirl the fully supplemented (complete) medium to ensure a homogeneous mixture. The complete medium is now ready to use.

***Note*: Although each component in this kit is supplied sterile, it is strongly recommended to filter the fully supplemented (complete) medium.**

Preparation of the MSC Adipogenic Differentiation Medium B (Maintenance Medium)

1. Prior to use, thaw the MSC-Qualified Fetal Bovine Serum at 2-8°C overnight or until completely thawed. Gently swirl the bottle to ensure homogeneity. The serum has been heat-inactivated and is ready to use after thawing.

***Note*: The thawed serum may contain some flocculent precipitates. The presence of these substances in serum does not alter the performance characteristics of the product. It is not recommended to filter the serum to remove these precipitates. Doing so may result in the loss of some serum nutrients.**

2. About 30 minutes prior to use, thaw Insulin, Penicillin-Streptomycin solution, and Glutamine solution at room temperature. Gently invert the vials to ensure homogeneity.

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

3. Disinfect the external surfaces of the bottles/vials for every component in the kit with 70% v/v ethanol. Allow ethanol to evaporate.
4. Aseptically open the bottles/vials inside a laminar flow hood.
5. Transfer the entire amount of MSC-Qualified Fetal Bovine Serum, Penicillin-Streptomycin solution, and Glutamine solution into the MSC Adipogenic Differentiation Basal Medium B.
6. Rinse each vial with a small amount of basal medium B. Subsequently transfer the entire rinse medium back into the bottle of basal medium B.
7. Transfer the entire amount of Insulin into MSC Adipogenic Differentiation Basal Medium B. Rinse the vial with a small amount of basal medium B. Subsequently transfer the entire rinse medium back into the bottle of basal medium B.
8. Repeat step 7 several times.
9. Gently swirl the fully supplemented (complete) medium to ensure a homogeneous mixture. The complete medium is now ready to use.

***Note*: Although each component in this kit is supplied sterile, it is strongly recommended to filter the fully supplemented (complete) medium.**

ADIPOGENESIS PROTOCOL:

***Note*: The protocol listed below is for 6-well tissue culture plates.**

1. Culture the OriCell™ MSCs in the OriCell™ Mesenchymal Stem Cell Growth Medium at 37°C in a 5% CO_2 humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with 0.25%Trypsin-0.04%EDTA (Cat. No. TEDTA-1000).
3. Reseed the MSCs in growth medium at $2x10^4$ cells/cm^2 in a 6-well tissue culture plate with a medium volume of 2 mL per well.
4. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
5. Feed the cells every three days until they are 100% confluent or post-confluent. Induction of adipogenic differentiation at post-confluency is strongly recommended.
6. When the cells are 100% confluent or post-confluent, carefully aspirate off the spent growth medium from the wells and add 2 mL of OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation medium A (induction medium) per well.
7. Three days later, change the medium to OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation medium B (maintenance medium) by completely replacing the spent medium A.
8. 24 hours later, change the medium back to MSC Adipogenic Differentiation medium A.
9. To optimally differentiate MSCs into adipogenic cells, repeat the cycle of induction and maintenance at least three times.
10. After three to five cycles of induction and maintenance, culture the cells in OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation medium B for an additional 4-7

days until the lipid droplets are big, round enough. During this days period, change the medium every three days.

OIL RED O STAINING ANALYSIS:

1. After the cells have differentiated, remove the MSC Adipogenic Differentiation Medium from the wells and rinse with 1x phosphate-buffered saline (PBS). Fix cells with 2 mL of 4% formaldehyde solution for 30 minutes.
2. Rinse wells twice with 1x PBS and stain cells with 1 mL of oil red O working solution (3:2 dilution with distilled water and filter with filter paper) for 30 minutes.
3. Rinse wells 2-3 times with 1x PBS.
4. Cells can now be visualized and analyzed under a microscope.

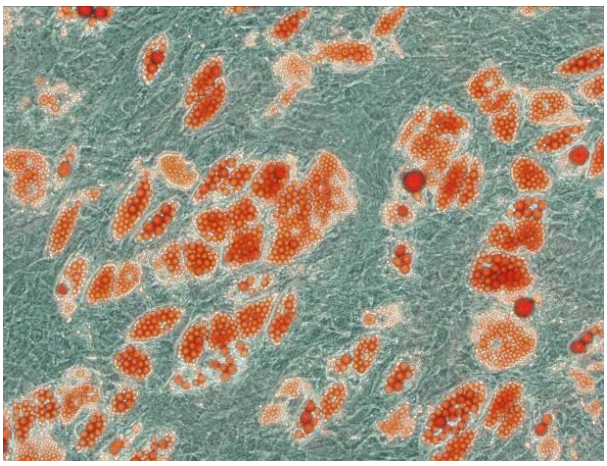

Fig.1 OriCell™ Human MSCs are differentiated into adipocytes and are stained with oil red O.

STABILITY AND STORAGE:

All products should be stored in the dark. MSC Adipogenic Differentiation Basal Medium A and B are stable at 2-8°C for up to one year. 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 up to one month 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 Adipogenic Differentiation Medium has been tested for performance on MSCs. The standard evaluation includes:

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

- Osmolality
- Endotoxin

RELATED PRODUCTS:

Product	Catalog Number
OriCell™ Mesenchymal Stem Cell Growth Medium	GUXMX-90011
OriCell™ Human Mesenchymal Stem Cell Growth Medium	HUXMX-90011
OriCell™ Mouse Mesenchymal Stem Cell Growth Medium	MUXMX-90011
SCTS™ Super MSC™ Human MSC Serum-Free Medium	HUXMX-90061
0.25%Trypsin-0.04%EDTA	TEDTA-10001
OriCell™ Human Mesenchymal Stem Cells	HUXMA-01001
OriCell™ Wistar Rat Mesenchymal Stem Cells	RAWMX-01001
OriCell™ SD Rat Mesenchymal Stem Cells	RASMX-01001
OriCell™ F344 Rat Mesenchymal Stem Cells	RAFMX-01001
OriCell™ Rabbit Mesenchymal Stem Cells	RBXMX-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