cyagen We help you discover life

User Manual

OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium

Cat. No. GUXMX-90021

PRODUCT DESCRIPTION:

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

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) Osteogenic Differentiation Basal Medium (Cat. No. GUXMX-90021)	175 mL
Mesenchymal Stem Cell (MSC)-Qualified Fetal Bovine Serum (Cat. No. GUXMX-05001)	20 mL
Penicillin-Streptomycin	2 mL
Glutamine	2 mL
Ascorbate	400 µL
β-Glycerophosphate	2 mL
Dexamethasone	20 µL
Alizarin Red S	10 mL

INSTRUCTIONS:

Preparation of the Complete 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 Ascorbate, β-Glycerophosphate, Penicillin-Streptomycin solution, and Glutamine solution at room temperature. Gently invert the vials several times to ensure homogeneity.
3. About 10 minutes prior to use, thaw Dexamethasone at room temperature.

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

4. Disinfect the external surfaces of the bottles/vials for every component in the kit with 70% v/v ethanol. Allow ethanol to evaporate.
5. Aseptically open the bottles/vials inside a laminar flow hood.
6. Transfer the entire amount of Ascorbate, β-Glycerophosphate, MSC-Qualified Fetal Bovine Serum, Penicillin-Streptomycin solution, and Glutamine solution into the MSC Osteogenic Differentiation Basal Medium.
7. Rinse each vial/bottle with a small amount of basal medium. Subsequently transfer the rinse medium back into the bottle of basal medium.
8. To transfer the entire amount of Dexamethasone, add 0.5 mL medium to the vial, mix by pipetting, and then transfer the entire mixture back into the bottle of basal medium.
9. Repeat step 8 several times.
10. Gently swirl the fully supplemented (complete) medium to ensure a homogenous 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.**

GELATIN COATING OF TISSUE CULTURE VESSELS:

1. Add enough 0.1% gelatin solution into the culture vessel to completely cover its base.
2. Swirl until the gelatin solution evenly coats the entire vessel base. Let it sit for at least 30 minutes at room temperature.
3. Aspirate off all of the gelatin solution and allow the residual amount to evaporate by leaving the vessel sitting open inside the laminar flow hood/biological safety cabinet for no more than 30 minutes.
4. Enclose the culture vessel once it has dried.

OSTEOGENESIS PROTOCOL:

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

1. Culture the OriCell™ MSCs in OriCell™ MSC Growth Medium at 37°C in a 5% CO2 humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with 0.25%Trypsin-0.04%EDTA (Cat. No. TEDTA-10001).
3. Reseed the MSCs in the growth medium at **2×10^4 cells/cm^2** in a 6-well tissue culture plate pre-coated with 0.1% gelatin solution.
4. Incubate the cells at 37°C inside a 5% CO_2 humidified incubator.
5. When cells are approximately 60-70% confluent, carefully aspirate off the growth medium from each well and add 2 mL of OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium.
6. Feed cells every three days for 2-4 weeks by completely replacing the medium with fresh OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium (pre-

warmed to 37°C).

7. After 2-4 weeks of differentiation, cells can be fixed and stained with alizarin red S.

***Note*: To prevent osteoblasts from detaching, it is recommended to change half of the medium every two days before analysis.**

ALIZARIN RED S STAINING ANALYSIS:

1. After the cells have differentiated, remove the osteogenic 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. Stain the cells with 1 mL alizarin red S working solution for 3-5 minutes.
3. Rinse wells 2-3 times with 1x PBS.
4. Cells can now be visualized and analyzed under a microscope.

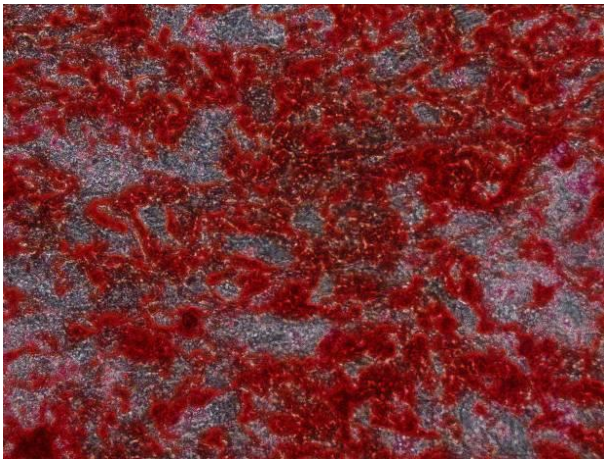

Fig. 1 OriCell™ Human MSCs are differentiated into osteocytes and are stained with alizarin red S.

STABILITY AND STORAGE:

All products should be stored in the dark. MSC Osteogenic Differentiation Basal Medium and alizarin red S 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 Osteogenic 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™ SuperMSC™ 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™ C57BL/6 Mesenchymal Stem Cells	MUBMX-01001
OriCell™ BALB/c Mesenchyal Stem Cells	MUCMX-01001

Cyagen Biosciences reserves all rights on the technical documents of its OriCell™ cell culture products. No part of this document may be reproduced or adapted for other purposes without written permission from Cyagen Biosciences.