

OriCell Rhesus Monkey Mesenchymal Stem Cell

Osteogenic Differentiation Medium

Catalog No. MKRMA-90021

Product Description:

Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Medium consists of optimized Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Basal Medium, pre-selected Fetal Bovine Serum and supplements. This product has been developed for the optimal differentiation of Rhesus Monkey Mesenchymal Stem Cells (Cat. No. MKRMA-01001) into osteoblasts.

The product is intended for laboratory research use only, not for drug, house hold, or other uses.

Kit Components:

Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Basal Medium (Cat. No. MKRMA-03021-175)	175 mL
Mesenchymal Stem Cell-Qualified Fetal Bovine Serum (Cat. No. MKRMA -05001-20)	20 mL
Penicillin-Streptomycin	2 mL
Glutamine	2 mL
Ascorbate	400 µL
β-Glycerophosphate	2 mL
Dexamethasone	20 µL

Instructions:

Preparation of Complete Medium

1. Prior to use, thaw Mesenchymal Stem Cell-Qualified Fetal Bovine Serum under refrigeration (2 to 8°C) over night or until completely thawed. Gently swirl the bottle to ensure homogeneity.

Mesenchymal Stem Cell-Qualified Fetal Bovine 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.

Note: Centrifuge the vials briefly at low speed (5,000g) before removing the caps to ensure recovery of entire content.

3. About 10 minutes prior to use, thaw Dexamethasone at room temperature.

Note: Centrifuge the vial briefly at low speed (5,000g) before removing the cap to ensure recovery of entire content.

4. Disinfect with 70% v/v ethanol the external surfaces of the bottles/vials for every component in the kit. Allow ethanol to evaporate away.
5. In a laminar flow hood aseptically open the bottles/vials.
6. Transfer the entire amount of Ascorbate, β -Glycerophosphate, Mesenchymal Stem Cell-Qualified Fetal Bovine Serum, Penicillin-Streptomycin solution and Glutamine solution into Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Basal Medium.
7. Rinse each vial/bottle with the medium and transfer the rinse medium back to the bottle of basal medium.
8. To transfer the entire amount of Dexamethasone, add 0.5 mL medium to the vial, mix by pipeting and then transfer the mixture back to the bottle of basal medium as much as possible.
9. Repeat step 8 several times.
10. 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.

Gelatin Coating of Tissue Culture Vessels

1. Add sufficient 0.1% Gelatin Solution into the culture vessel to completely cover its base.
2. Swirl 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. Put lid back once the surface is dry.

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

1. Rhesus Monkey Mesenchymal Stem Cells are cultured in Rhesus Monkey Mesenchymal Stem Cell Growth Medium (Cat. No. MKRMA-90011) (growth medium thereafter) at 37°C in a 5% CO₂ humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with Trypsin-EDTA (Cat. No. TEDTA-10001-100).
3. Rhesus Monkey Mesenchymal Stem Cells are replated in growth medium at 3×10^4 cells/cm² in 6-well tissue culture plates pre-coated with Gelatin Solution.
4. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
5. After 24 hours, carefully aspirate off the growth medium from each well and add 2 mL Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Medium.
6. Refeed cells every 3 days for 2-3 weeks by completely replace the medium with fresh Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Medium.
7. After 2-3 week differentiation, cells can be fixed and stained with Alizarin red.

Alizarin Red Stain Analysis

1. After differentiating, remove Osteogenic Differentiation Medium from well and rinse with 1×PBS. Fix cells with 2ml of 4% formaldehyde solution for 30 minutes.
2. Then rinse wells twice with 1×PBS and stain cells with 1ml alizarin red working solution for 3 to 5 minutes.
3. Rinse wells 2-3 times with 1×PBS, then visualize under light microscope and capture images.

Stability/Storage:

All products should be stored in the dark.

Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Basal Medium is stable at 2 to 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 to 8°C.

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

Quality Control:

Rhesus Monkey Mesenchymal Stem Cell Osteogenic Differentiation Medium is performance tested on Rhesus Monkey Mesenchymal Stem Cells.

Standard evaluation includes:

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

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