cyagen | OriCell

User Manual

OriCell™ Osteogenic Differentiation Medium For Mouse Bone Marrow Mesenchymal Stem Cells

Catalog No. MUXMX-90021

We help you discover life

Introduction

OriCell™ Osteogenic Differentiation Medium For Mouse Bone Marrow Mesenchymal Stem Cells is specially formulated and developed by our R&D team. It consists of an optimized basal medium, fetal bovine serum (FBS) and essential supplements, tailored to induce the osteogenic differentiation of mouse bone marrow mesenchymal stem cells (BMSCs).

Extensive data have demonstrated that this medium consistently facilitates the stable and efficient differentiation of these cells into osteoblasts.

Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences."

Product Information

Components	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture	BLDM-03011	177 mL	4 °C
OriCell™ Fetal Bovine Serum (Superior-Quality)	FBSSR-01021	20 mL	-20 °C
OriCell™ Supplements For Mouse Bone Marrow Mesenchymal Stem Cells Osteogenic Differentiation	MUXMX-04021	3 mL	-20 °C
Alizarin Red S Solution (pH = 5.1 ~ 5.3)	ALIR-10001	10 mL	4 °C
Gelatin	GLT-11301	10 mL	4 °C

QC

- Pass the detection of bacteria, fungi, mycoplasma and endotoxins.
- Pass the detection of osmotic pressure and pH.
- Pass the detection of product quality.

Please refer to "COA" for details.

General Handling Principles

1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual instructions. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Ensure proper storage and use. Store all components according to specified conditions and use them promptly to ensure optimal performance.
4. Aliquot for long-term storage. If the entire volume will not be used up immediately, prepare the medium in batches according to the specified component volume ratios and store in aliquots.

Product Stability and Storage Conditions

1. All components must be stored away from light.
2. The basal medium, Alizarin Red S solution and gelatin have a shelf life of 1 year and should be stored at 4 °C. Other components have a shelf life of 2 years and should be stored at -20 °C.
3. Once prepared, the medium has a shelf life of 1 month when stored at 4 °C. The shelf life may be extended up to a maximum of 45 days, provided that culture conditions remain stable, the container is properly sealed, and repeated temperature fluctuations are avoided.
4. Use all components before the expiration dates. Expired components may severely compromise culture performance.

Preparation of Complete Medium

Materials Required

- OriCell™ Osteogenic Differentiation Medium For Mouse Bone Marrow Mesenchymal Stem Cells (Cat. No.: MUXMX-90021)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil and other light-avoiding materials

Steps

1. At least 6 hours before preparation, place the OriCell™ Fetal Bovine Serum (Cat. No.: FBSSR-01021) in a refrigerator at 4 °C to allow it to thaw completely.

Note: Flocs (primarily composed of fibrin) may appear in thawed serum and will not affect product performance. Removal of flocs is generally unnecessary unless the cell culture system demands a high degree of purification.

2. At least 30 minutes before preparation, place the OriCell™ Supplements For Mouse Bone Marrow Mesenchymal Stem Cells Osteogenic Differentiation (Cat. No.: MUXMX-04021) in a refrigerator at 4 °C until it is completely thawed.
3. Mix the reagents by inverting the tube several times or gently flicking the bottom of the tube.
4. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
5. Add all of the serum (Cat. No.: FBSSR-01021) and the supplement (Cat. No.: MUXMX-04021) to OriCell™ Basal Medium For Cell Culture (Cat. No.: BLDM-03011).
6. Rinse each bottle and tube with a small volume of basal medium, then transfer the washings back to the basal medium bottle to maximize recovery.
7. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.
8. Seal the bottle with Parafilm, wrap it in aluminum foil to protect from light, and label it with the product name, preparation date, and other relevant information.

Special Reminder

- If the entire medium will not be used up immediately, we recommend preparing in batches. Please prepare the required amount according to the volume ratio of each component in the kit. Any remaining components must be stored according to their respective storage conditions and should not be subjected to multiple freeze-thaw cycles.
- All components in the OriCell™ Osteogenic Differentiation Medium For Mouse Bone Marrow Mesenchymal Stem Cells are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilization again. If there is a risk of contamination during the preparation process, the medium can be filtered and sterilized.

- The prepared medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Osteogenic Differentiation Medium For Mouse Bone Marrow Mesenchymal Stem Cells (Cat. No.: MUXMX-90021)
- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)

Steps

Note:

1) This protocol uses a 6-well plate as an example. Please select an appropriate culture vessel based on your specific needs.

2) To minimize cell detachment or floating and ensure optimal adherence during induction, it is recommended to coat the culture vessel with 0.1% gelatin.

3) Pre-warm the induction medium to 37 °C before use.

1. Add 1 mL of 0.1% gelatin solution to each well of the 6-well plate and gently swirl to ensure even coverage.
2. Place the gelatin-coated plate on an ultraclean bench (laminar flow hood) or a CO_2 incubator for at least 30 minutes.
3. After 30 minutes, aspirate the gelatin solution. The plate can then be used immediately for cell seeding or air-dried before use.

4. Seed the cells at a density of 2×10^4 cells/cm^2 and add 2 mL of regular complete medium to each well.
5. Incubate the cells at 37 °C, 5% CO_2, and saturated humidity.
6. When the cells reach 70% confluence, carefully aspirate the complete medium and add 2 mL of osteogenic differentiation medium (Cat. No.: MUXMX-90021) to each well.
7. Replace the medium with fresh differentiation medium every 3 days.
8. After 2–4 weeks of induction, perform Alizarin Red staining, depending on cell morphology and growth status.

   Note: **To prevent cell detachment and loss of calcium nodules, it is recommended to perform a half-medium change every 2 days once significant calcium nodules appear during the induction process.**

Alizarin Red Staining Analysis

Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001))
- 4% Paraformaldehyde or 10% Formalin
- OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001)

Steps

Note:

(1) Handle all procedures gently to prevent detachment of calcium nodules.

(2) Bring OriCell™ Alizarin Red S Solution to room temperature before use.

(3) Ensure the presence of calcium nodules before staining.

(4) If staining is insufficient, the incubation time may be extended as appropriate.

1. After the induction process is complete, aspirate the differentiation medium from the 6-well plate and wash each well gently 2–3 times with 1× PBS.
2. Add 2 mL of 4% paraformaldehyde solution or 10% formalin solution to each well and fix the cells at room temperature for 30 minutes.
3. Aspirate the fixative and gently wash 2–3 times with 1× PBS to ensure complete removal.
4. Add 2 mL of OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001) to each well and incubate for 5–10 minutes at room temperature.
5. Aspirate the staining solution and rinse the wells 2–3 times with 1× PBS to ensure thorough removal of excess stain.
6. Add 2 mL of 1 × PBS to each well and observe the staining results under a microscope.
7. Seal the plate with Parafilm and protect from light. Stained plates can be stored at 4 °C for up to 2 weeks.

Alizarin Red Staining Results