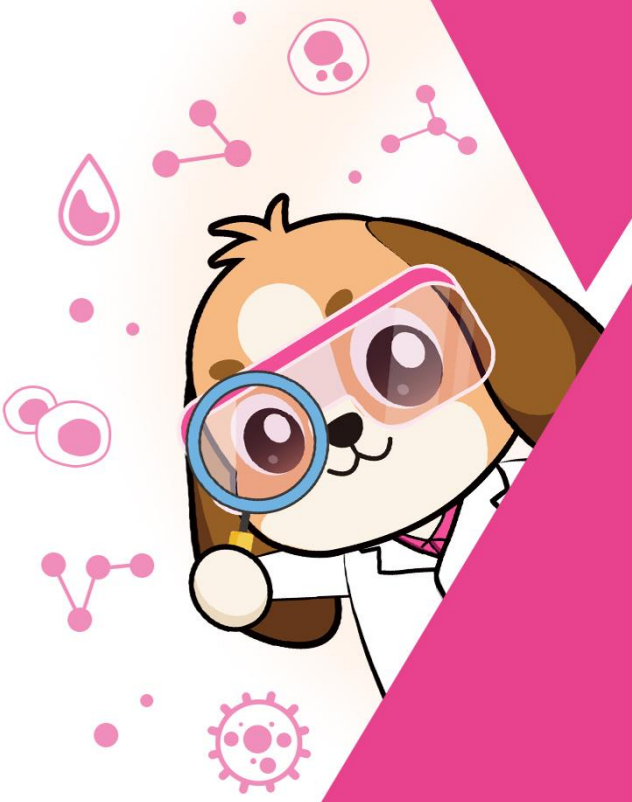
cyagen | OriCell™

User Manual

OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells



Catalog No. MUXMT-90021

We help you discover life

Introduction

OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells, developed by OriCell™ R&D team, contains a basic medium suitable for the growth of MC3T3-E1 cells, OriCell™ Premium Fetal Bovine Serum, and a supplement for osteogenic differentiation of MC3T3-E1 cells.

This product is suitable for osteogenic induction and differentiation of mouse MC3T3-E1 cells. Extensive data demonstrate that this product can induce these cells to differentiate efficiently and stably into osteoblasts.

Note: **This product is only provided for further scientific research. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic journals, please indicate "OriCell™ + Catalog Number, from Cyagen Biosciences (Guangzhou) Inc."

Product Information

Components	Catalog Number	Volume
OriCell™ Basal Medium For Cell Culture	BLDM-03011	177 mL
OriCell™ Fetal Bovine Serum（Superior-Quality）	FBSSR-01021	20 mL
OriCell™ Supplements For Mouse MC3T3-E1 Cells Osteogenic Differentiation	MUXMT-04021	3 mL
Alizarin Red S Solution（pH=5.1~5.3）	ALIR-10001	10 mL
Gelatin	GLT-11301	10 mL

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 reference "COA" for details.

General Handing Principles

1. Ensure that all equipment is kept clean and tidy.
2. Standard operation method. Please operate according to the method described in the product manual.
3. The ingredients should be properly stored in accordance with the storage conditions and used as soon as possible.
4. If complete medium cannot be used in a short period of time, it should be prepared in batches according to the volume ratio of each component in the kit and stored in aliquots.

Product Stability and Storage Conditions

1. All ingredients must be kept in dark place.
2. The basal medium must be stored in a refrigerator at 4°C for a period of 1 year. Other components must be stored at -20°C for a period of 2 years.

3. The prepared complete medium can be stored at 4°C for a period of 1 month. If the culture conditions are stable, the container has great sealing performance, and there is no alternation of hot and cold condition, the period of using can be appropriately extended, but not exceed 45 days.
4. Please use all products within the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells (Cat. No. : MUXMT-90021)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil paper and other light-avoiding materials

Steps

1. At least 6 hours before preparation, place the OriCell™ Superior Fetal Bovine Serum in a refrigerator at 4°C to allow it thaw completely.

   Note: **Flocs may appear in the thawed serum, the main component of fibrin, which will not affect the product performance. Unless the cell culture system is required for a high degree of purification, we do not recommend removing flocs.**
2. At least 30 minutes before preparation, place the OriCell™ Supplements For Mouse MC3T3-E1 Cells Osteogenic Differentiation in a refrigerator at 4°C until it is completely thawed.
3. Turn upside to down or flick the reagent tube to mix the reagent.
4. Use 75% ethanol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.

5. Add all the serum and supplements to OriCell™ Basal Medium For Cell Culture (Cat. No.: BLDM-03011).
6. Take a little amount of basal medium, wash each bottle and tube, and add all the ingredients to the basal medium as much as possible.
7. Tighten the cap of the basal medium bottle and shake it gently and thoroughly.
8. Seal the bottle with parafilm, wrap the bottle with aluminum foil paper, and mark the name, preparation date and other information.

Special Reminder

- **If complete medium will not be used immediately, we recommend to preparing in batches. Please prepare the required amount according to the ratio of each component in the kit, but the remaining components must be stored in accordance with their respective storage conditions and not be frozen and thawed multiple times.**
- **All components in the OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 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 complete medium can be filtered and sterilized.**
- **The prepared osteogenic differentiation medium should be divided into small aliquots to avoid repeated warm bathing and refrigeration of the whole bottle of medium.**

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells (Cat. No.: MUXMT-90021)
- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No. PBS-10001)
- OriCell™ Alizarin Red S Solution (Cat. No.: ALIR-10001)

Steps

Note:

1) This operating procedure takes a 6-well plate as an example, please select a suitable culture container according to your experiments.

2) In order to avoid the cells floating, we recommended to use gelatin to coat the culture container.

3) The induction medium needs to be preheated to 37°C before use.

1. Add 1 mL of 0.1% gelatin to the 6-well plate and shake it completely cover the bottom of each well.
2. Place the 6-well plate covered with 0.1% gelatin in the ultraclean bench or CO_2 incubator for at least 30 minutes.
3. After 30 minutes, suck off the gelatin to inoculate cells, or wait for the 6-well plate to dry before inoculation.
4. Inoculate the MC3T3-E1 cells in the 6-well plate at a cell density of 2×10^4 cells/cm^2, and add 2 mL of ordinary complete medium to each well.
5. The cells are cultured in a CO_2 incubator at 37°C, 5% CO_2, with saturated humidity.
6. When the cell density reaches 70%, please add 2 mL OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells to the 6-well plate carefully.
7. Replace with fresh OriCell™ Osteogenic Differentiation Medium For Mouse MC3T3-E1 Cells every 3 days.
8. After 2 to 4 weeks of induction, stain with OriCell™ Alizarin Red S Solution depending on the morphological changes and growth of the cells.

   Note: **To prevent the loss of osteoblasts and calcium nodules, we recommended to change the fluid in half each two days after obvious calcium nodules could be found in the process of osteogenesis**

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) To prevent calcium nodules falling off, all operations should be performed as gently as possible.

2) Please return to room temperature before using OriCell™ Alizarin Red S Solution. If there is no effective dyeing, the dyeing time can be extended appropriately.

3) Make sure calcium nodules appear before performing staining.

1. After the osteogenic differentiation is completed, aspirate the complete osteogenic differentiation medium in the 6-well plate, and wash with 1×PBS for 2~3 times gently .
2. Add 2 mL of 4% Paraformaldehyde (or 10% Formalin) to each well and fix cells at room temperature for 30 min.
3. Aspirate the fixative and wash with 1×PBS 2~3 times gently to ensure that the fixative is thoroughly washed.
4. Add 2 mL of alizarin red staining to each well and stain for 5~10 min at room temperature.
5. Aspirate the alizarin red staining , and wash with 1×PBS for 2~3 times to fully wash off the excess staining solution.
6. Add 2 mL 1 × PBS to each well and place the culture plate under a microscope to observe the results of osteogenic staining.
7. After the dyed 6-well plate is encapsulated with parafilm, it can be stored at 4°C for 2 weeks.

The Alizarin Red S Solution Staining Effect