cyagen | OriCell™

User Manual

OriCell™ Adipogenic Differentiation Medium For Mouse MC3T3-E1 Cells

Catalog No. MUXMT-90031

We help you discover life

Introduction

OriCell™ Adipogenic Differentiation Medium For Mouse MC3T3-E1 Cells, carefully developed by the OriCell™ R&D team, contains a basic medium suitable for the growth of mouse MC3T3-E1 cells, fetal bovine serum and various additives required for inducing cell differentiation.

This product is suitable for adipogenic induction and differentiation of Mouse MC3T3-E1 cells. Extensive cell culture data have demonstrated that it can reliably and efficiently induce these cells to differentiate into adipocytes.

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 of Solution A	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 Adipogenic Differentiation A-I	MUXMT-04031-a1	3 mL
OriCell™ Supplements For Mouse MC3T3-E1 Cells Adipogenic Differentiation A-II	MUXMT-04031-a2	200 µL

Components of Solution B	Catalog Number	Volume
OriCell™ Basal Medium For Cell Culture	BLDM-03011	90 mL
OriCell™ Fetal Bovine Serum (Superior-Quality)	FBSSR-01021	10 mL
OriCell™ Supplements For Mouse MC3T3-E1 Cells Adipogenic Differentiation B	MUXMT-04031-b	200 μL

Other Components	Catalog Number	Volume
OriCell™ Oil Red O Solution (pH=2.0~2.2)	OILR-10001	5 mL
OriCell™ 0.1% Gelatin Solution	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 refer to "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 should be stored in a refrigerator at 4 °C for a period of 1 year. Other components should 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 before the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Adipogenic Differentiation Medium For Mouse MC3T3-E1 Cells (Cat. No.: MUXMT-90031)
- 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

Preparation of Solution A

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

   Note: **There may be floccules in the thawed serum, mainly composed of fibrin, which will not affect product performance. If the required purity of the cell culture system is not extremely high, filtration or centrifugation to remove flocs is not recommended.**

2. At least 30 minutes before preparation, place OriCell™ Supplements For Mouse MC3T3-E1 Cells Adipogenic Differentiation A-I (Cat. No.: MUXMT-04031-a1) in a refrigerator at 4°C. Keep OriCell™ Supplements For Mouse MC3T3-E1 Cells Adipogenic Differentiation A-II (Cat. No.: MUXMT-04031-a2) at room temperature until completely thawed.

   Note: **It is normal for supplement A-II (Cat. No.: MUXMT-04031-a2) to develop granular precipitation after thawing. This can be redissolved by pipetting repeatedly after a short 37°C water bath.**

3. Invert or flick the reagent tube to mix the reagent thoroughly.
4. Briefly centrifuge the reagent tube of supplement A-II to ensure that the reagent is concentrated at the bottom for easy collection.
5. Carefully wipe the outer packaging of all components with 75% ethanol. Open the packages inside a clean bench.
6. Add all serum, supplement A-I and supplement A-II to OriCell™ Basal Medium (Cat. No.: BLDM-03011).

   Note: **To ensure proper dissolution, preheat the basal medium to 37°C. Otherwise, supplement A-II may precipitate when cold.**

7. Take a small amount of basal medium to rinse each bottle and tube, and add all rinsed ingredients to the basal medium as much as possible.
8. Tighten the cap of the basal medium bottle and shake it gently and thoroughly.
9. Seal the bottle opening with parafilm, wrap the bottle in aluminum foil, and label it with the product

name, preparation date, and other relevant information.

Preparation of Solution B

1. At least 6 hours before preparation, put the OriCell™ Fetal Bovine Serum (Superior-Quality) (Cat.No. FBSSR-01021) in a refrigerator at 4°C to allow it to thaw completely.
2. At least 30 minutes before preparation, place the OriCell™ Supplements For Mouse MC3T3-E1 Cells Adipogenic Differentiation B (Cat. No.: MUXMT-04031-b) in a refrigerator at 4°C until completely thawed.
3. Invert or flick the reagent tube to mix the reagent thoroughly.
4. Briefly centrifuge the reagent tube of supplement B to ensure that the reagent is concentrated at the bottom for easy collection.
5. Carefully wipe the outer packaging of all components with 75% ethanol. Open the packages inside a clean bench.
6. Add all serum and supplement B to OriCell™ Basal Medium (Cat. No.: BLDM-03011).
7. Take a small amount of basal medium to rinse each bottle and tube, and add all rinsed ingredients to the basal medium as much as possible.
8. Tighten the cap of the basal medium bottle and shake it gently and thoroughly.
9. Seal the bottle opening with parafilm, wrap the bottle in aluminum foil, and label it with the product name, preparation date, and other relevant information.

Special Reminder

- **If the medium will not be fully used immediately, we recommend preparing in batches. Please prepare the required amount according to the 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™ Adipogenic 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 adipogenic differentiation medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Adipogenic Differentiation Medium For Mouse MC3T3-E1 Cells (Cat. No.: MUXMT-90031)
- 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 six-well plate as an example. Please select an appropriate culture vessel based on your specific needs.

2) To minimize cell detachment and floating and to improve cell adherence during induction, it is recommended to coat the culture vessel with gelatin.

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

1. Add 1 mL of OriCell™ 0.1% Gelatin Solution to each well of the six-well plate and gently shake to ensure even coverage.
2. Place the six-well plate coated with OriCell™ 0.1% Gelatin Solution on the ultra-clean bench or CO_2 incubator for at least 30 minutes.
3. After 30 minutes, aspirate the gelatin solution. You may inoculate cells immediately or allow the plate to air dry before inoculation.

4. Seed the mouse MC3T3-E1 cells intended for induction into a six-well plate at a density of 2×10^4 cells/cm^2, and add 2 mL of standard complete medium to each well.
5. Culture the cells in a CO_2 incubator at 37°C, 5% CO_2, and saturated humidity.
6. When cells reach 100% confluence, carefully aspirate the complete medium and add 2 mL of solution A to each well.
7. After 3 days of induction, aspirate solution A in the six-well plate and add 2 mL of solution B
8. After 1 day, aspirate solution B and switch back to solution A for induction.
9. Alternate between solution A and solution B as described, monitoring cell morphology daily. If cells shrink or die during solution A induction, promptly switch to solution B until cell health recovers.

   Note:

   1) Solution A induces the formation of lipid droplets, while solution B maintains the formed lipid droplets and promotes their accumulation.

   2) Under normal circumstances, the use of "A solution for 3 days, B solution for 1 day" can smoothly induce cell adipogenesis.

   3) Due to variability among cell types and batches, adjust the ratio and duration of Solutions A and B flexibly according to cell response.

10. Repeat the induction and maintenance cycle until sufficient lipid droplets of appropriate size have formed and are ready for staining.

Oil Red O Staining Analysis

Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- 4% Paraformaldehyde Solution or 10% Formalin Solution
- OriCell™ Oil Red O Solultion (Cat. No.: OILR-10001)

Steps

Note: **To prevent lipid droplets from detaching, handle all steps as gently as possible.**

1. After the adipogenic induction and differentiation, aspirate the complete adipogenic differentiation medium in the six-well plate, and gently wash 2 to 3 times with 1× PBS.
2. Add 2 mL of 4% paraformaldehyde solution (or 10% formalin) to each well and fix for 30 minutes at room temperature.
3. Prepare the working solution by mixing OriCell™ Oil Red O Solultion and distilled water at a ratio of 3:2. After thorough mixing, centrifuge the mixture at 250 × g for 4 minutes, and use the supernatant for staining.
4. Aspirate the fixative, then gently wash 2 to 3 times with 1× PBS to ensure the fixative is thoroughly removed.
5. Add 2 mL of the working solution to each well and incubate for 30 minutes at room temperature.
6. Aspirate the working solution, then gently wash 2 to 3 times with 1× PBS to thoroughly remove the staining solution.
7. Add 2 mL of 1× PBS to each well, and observe the lipid staining under a microscope.
8. After staining, seal the six-well plate with parafilm and store it at 4°C for no longer than one week. Prolonged storage may cause lipid droplets to fuse, altering their original stained morphology.

The Effect of Oil Red O Solution Staining