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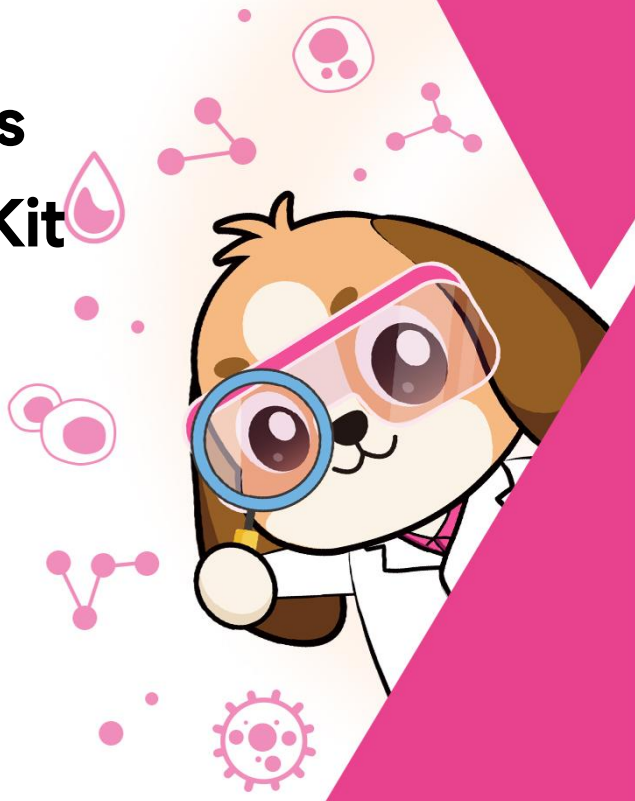
User Manual

OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation Kit



Catalog No. MUXTL-90031

We help you discover life

Introduction

The OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation Kit was carefully developed by the OriCell™ R&D team includes a basic medium suitable for the growth of Mouse 3T3-L1 Cells, OriCell™ premium fetal bovine serum and various additives required for inducing cell differentiation.

This product is suitable for adipogenic induction and differentiation of Mouse 3T3-L1 Cells. A large number of cell culture data verify that this product can stably and efficiently induce the above-mentioned 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™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation A-I	MUXTL-04031-a1	3 mL
OriCell™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation A-II	MUXTL-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™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation B	MUXTL-04031-b	200 μL

Other components	Catalog Number	Volume
Oil Red O Solultion（pH=2.1）	OILR-10001	5 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 seriously affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation Kit
- 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 in a refrigerator at 4°C to completely melt.

   Note: **There may be floccules in the thawed serum, the main component of which is fibrin, which will not affect the effect of the product. If the required purity of the cell culture system is not extremely high, we do not recommend filtration or centrifugation to remove flocs.**

2. At least 30 minutes before preparation, place OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation Supplement A-I in a refrigerator at 4°C; OriCell™ Mouse 3T3-L1 Cells Adipogenesis Induction Differentiation Additive A-II is placed at room temperature until completely melted.

   Note: **After the melted additive A-II appears granular precipitation, it is a normal phenomenon. It can be re-dissolved by pipetting repeatedly after a short 37°C water bath.**

3. Turn upside down or flick the reagent tube to mix the reagent.
4. Centrifuge the additive A-II reagent tube briefly to ensure that the reagent is concentrated at the

bottom of the tube for collection.

5. Use 75% medical alcohol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.
6. Add all the serum, supplement A-I, and supplement A-II to OriCell™ Basal Medium.
   Note : **In order to ensure a good dissolution effect, please preheat the basic medium to 37°C, otherwise the additive A-II may precipitate out when cold.**
7. Take a small amount of basal medium, wash each bottle and tube, and add all the ingredients to the basal medium as much as possible.
8. Tighten the cap of the basal medium bottle, shake gently and thoroughly.
9. Seal the mouth of the bottle with parafilm, wrap the bottle with aluminum foil, and mark the name, preparation date and other information.

Preparation of Solution B

1. At least 6 hours before preparation, put the OriCell™ Fetal Bovine Serum in a refrigerator at 4°C to completely melt.
2. At least 30 minutes before preparation, place the OriCell™ Mouse 3T3-L1 Cells adipogenic differentiation supplement B in a refrigerator at 4°C until it is completely melted.
3. Turn upside to down or flick the reagent tube to mix the reagent.
4. Centrifuge the reagent tube of Additive B briefly to ensure that the reagents are concentrated at the bottom of the tube for easy collection.
5. Use 75% medical alcohol to carefully wipe the outer packaging of all ingredients. Open the package in the clean bench.
6. Add all the serum and supplement B to OriCell™ Basal Medium.
7. 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.
8. Tighten the cap of the basal medium bottle and shake it gently and thoroughly.
9. 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 cannot be used in the short term, 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™ Mouse MC3T3-E1 Cells Adipogenic Differentiation Kit are strictly controlled aseptic. 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 divided into small aliquots to avoid repeated warm bathing and refrigeration of the whole bottle of medium.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation Kit
- OriCell™ 0.1% Gelatin（Cat：GLT-11301）
- OriCell™ Phosphate-Buffered Saline (1×PBS)（Cat：PBS-10001）

Steps

Note：1）This operating procedure takes a six-well plate as an example, please choose a suitable culture container according to the actual situation;

2）In order to reduce the cells floating and not sticking to the wall during the induction process, it is 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 six-well plate and shake it to make it evenly cover the bottom of each well.
2. Place the six-well plate covered with 0.1% gelatin on the ultra clean 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 six-well plate to dry before inoculation.
4. Inoculate the Mouse 3T3-L1 Cells to be induced in a six-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, and saturated humidity.
6. When the cell confluence reaches 100%, carefully aspirate the complete medium in the well, and add 2 mL of medium A solution to the six-well plate.

7. After 3 days of induction, aspirate solution A in the six-well plate and add 2 mL solution B.
8. After maintaining for 1 day, aspirate solution B and switch to solution A for induction.
9. Solution A and solution B are used alternately, during which the cell status needs to be observed every day. If the cells shrink or die during the induction of solution A, please change to solution B in time until the cell state is restored.

   Note: **1) Solution A stimulates the formation of lipid droplets; solution B maintains the formed lipid droplets and promotes the increase of lipid droplets;**

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

   3) Various conditions may occur during the induction process of various types and batches of cells. Please adjust the ratio of A and B solution use flexibly.
10. Repeat the induction and maintenance process until there are enough lipid droplets of suitable size, ready for dyeing.

Oil Red O staining analysis

Materials Required

- OriCell™ Phosphate-Buffered Saline (1×PBS) (Cat: PBS-10001)
- 4% Paraformaldehyde solution or 10% Formalin solution
- Oil Red O Staining Solution

Steps

Note: **In order to prevent the lipid droplets from falling off, all operations should be as gentle as possible.**

1. After the adipogenic induction and differentiation, aspirate the complete adipogenic differentiation medium in the six-well plate, and wash gently with 1×PBS for 2 to 3 times.
2. Add 2 mL of 4% paraformaldehyde solution (or 10% formalin) to each well, fix for 30 min at room temperature.
3. According to the ratio, oil red O storage solution: distilled water=3:2, prepared as a working solution. After mixing, centrifuge at 250×g for 4 min, and use the supernatant.
4. Aspirate the fixative and wash gently with 1×PBS for 2 to 3 times to ensure that the fixative is thoroughly washed.
5. Add 2 mL of Oil Red O dye working solution to each well and dye for 30 min at room temperature.

6. Aspirate the oil red O staining solution, and gently wash with 1×PBS for 2 to 3 times to fully wash off the staining solution.
7. Add 2 mL 1×PBS to each well, and place the culture plate under a microscope to observe the lipid forming staining effect.
8. After dyeing, the six-well plate is sealed with parafilm and stored at 4°C, but not more than 1 week. The lipid droplets will fuse with each other and cannot maintain the state they were in when they were dyed.

The Oil Red O Staining Effect of OriCell™ Mouse 3T3-L1 Cells Adipogenic Differentiation