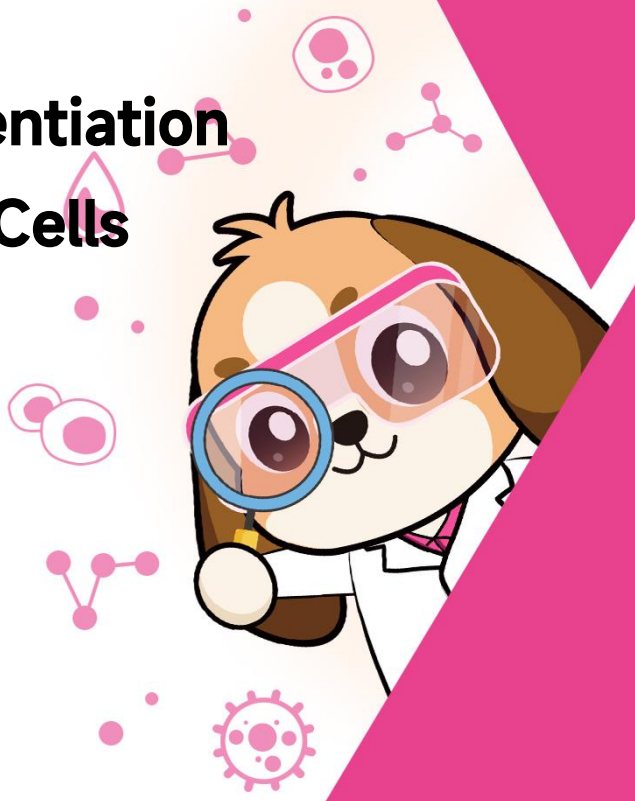
cyagen | OriCell™

User Manual

OriCell™ Adipogenic Differentiation Medium For Mouse 3T3-L1 Cells

Catalog No. MUXTL-90031



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Introduction

The OriCell™ Adipogenic Differentiation Medium For Mouse 3T3-L1 Cells, 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. Extensive cell culture data have demonstrated 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
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 seriously affect the cell culture effect.

Preparation of Complete Medium

Materials Required

- OriCell™ Adipogenic Differentiation Medium For Mouse 3T3-L1 Cells (Cat. No.: MUXTL-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 (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™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation A-I (Cat. No.: MUXTL-04031-a1) in a refrigerator at 4°C. Keep OriCell™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation A-II (Cat. No.: MUXTL-04031-a2) at room temperature until completely thawed.

   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. Carefully wipe the outer packaging of all ingredients with 75% ethanol. Open the package inside a clean bench.
6. Add all serum (Cat. No.: FBSSR-01021), supplement A-I (Cat. No.: MUXTL-04031-a1), and supplement A-II (Cat. No.: MUXTL-04031-a2) to OriCell™ Basal Medium (Cat. No.: BLDM-03011).

   Note: **In order to ensure a good dissolution effect, please preheat the basic medium to 37°C, otherwise the supplement 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 (Cat. No.: FBSSR-01021) in a refrigerator at 4°C to completely thaw.

2. At least 30 minutes before preparation, place the OriCell™ Supplement For Mouse 3T3-L1 Cells Adipogenic Differentiation B (Cat. No.: MUXTL-04031-b) 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. Centrifuge the reagent tube of supplement B briefly to ensure that the reagents are concentrated at the bottom of the tube for easy collection.
5. Carefully wipe the outer packaging of all ingredients with 75% ethanol. Open the package inside a clean bench.
6. Add all serum (Cat. No.: FBSSR-01021) and supplement B (Cat. No.: MUXTL-04031-b) to OriCell™ Basal Medium (Cat. No.: BLDM-03011).
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 the medium will not be used up 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 3T3-L1 Cells 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 aliquoted into small portions to avoid**

repeated freeze-thaw cycles.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Adipogenic Differentiation Medium For Mouse 3T3-L1 Cells (Cat. No.: MUXTL-9003)
- OriCell™ 0.1% Gelatin Solution (Cat No.: GLT-11301)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: 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 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: **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. Prepare the working solution by mixing OriCell™ Oil Red O Solultion (Cat. No.: OILR-10001) and distilled water at a 3:2 ratio. After thorough mixing, centrifuge at 250×g for 4 minutes and use the supernatant for staining.
4. Aspirate the fixative and gently wash the wells 2 to 3 times with 1× PBS to ensure complete removal of the fixative.
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 the wells 2 to 3 times with 1× PBS to thoroughly remove the stain.
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. Note that lipid droplets may fuse over time and will not retain their original stained morphology.

The Effect of Oil Red O Staining