cyagen | OriCell

User Manual

OriCell™ Adipogenic Differentiation Medium For Mouse Tendon Stem Cells

Catalog No. MUXTA-90031

We help you discover life

Introduction

OriCell™ Adipogenic Differentiation Medium for Mouse Tendon Stem Cells is specially formulated and developed by our R&D team. It consists of an optimized basal medium, premium fetal bovine serum (FBS), and essential growth supplements, all tailored to induce the adipogenic differentiation of mouse tendon stem cells.

Extensive cell culture data have demonstrated that this medium supports the stable and efficient differentiation of mouse tendon stem cells into adipocytes.

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 of Solution A	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™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation A-I	MUXTA-04031-a1	3 mL	-20 °C
OriCell™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation A-II	MUXTA-04031-a2	200 μL	-20 °C

Components of Solution B	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture	BLDM-03011	90 mL	4 °C
OriCell™ Fetal Bovine Serum (Superior-Quality)	FBSSR-01021	10 mL	-20 °C
OriCell™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation B	MUXTA-04031-b	200 μL	-20 °C

Other Components	Catalog Number	Volume	Storage
OriCell™ Oil Red O Solultion (pH=2.1)	OILR-10001	5 mL	4 °C
OriCell™ 0.1% Gelatin Solution	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, Oil Red O solultion and gelatin solution 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 Premix Solution

Materials Required

- OriCell™ Adipogenic Differentiation Medium For Mouse Tendon Stem Cells (Cat. No.: MUXTA-90031)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil 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: **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™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation A-I (Cat. No.: MUXTA-04031-a1) in a refrigerator at 4 °C to allow it to thaw completely. Meanwhile, keep OriCell ™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation A-II (Cat. No.: MUXTA-04031-a2) at room temperature until it is completely thawed.

   Note: **Granular precipitation may occur in Supplement A-II after thawing and is considered normal. These precipitates can be redissolved by brief incubation in a 37 °C water bath followed by repeated pipetting.**

3. Mix the reagents by inverting the tube several times or gently flicking the bottom of the tube.
4. Centrifuge the Supplement 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 components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
6. Add all serum (Cat. No.: FBSSR-01021), Supplement A-I (Cat. No.: MUXTA-04031-a1), and Supplement A-II (Cat. No.: MUXTA-04031-a2) to OriCell™ Basal Medium For Cell Culture (Cat. No.: BLDM-03011).

   Note: **To ensure complete dissolution, pre-warm the basal medium to 37 °C. Otherwise, Supplement A-II may precipitate if added to cold medium.**

7. 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.
8. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.
9. 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.

Preparation of Solution B

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.
2. At least 30 minutes before preparation, place the OriCell™ Supplement For Mouse Tendon Stem Cells Adipogenic Differentiation B (Cat. No.: MUXTA-04031-b) 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. Centrifuge the Supplement B 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 components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
6. Add all serum (Cat. No.: FBSSR-01021) and Supplement B (Cat. No.: MUXTA-04031-b) to OriCell™ Basal Medium For Cell Culture (Cat. No.: BLDM-03011).
7. 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.
8. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.
9. 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 Notes

- **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™ Adipogenic Differentiation Medium For Mouse Tendon 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™ Adipogenic Differentiation Medium For Mouse Tendon Stem Cells (Cat. No.: MUXTA-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 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 in a laminar flow hood (ultraclean bench) 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 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 plate and add 2 mL of Solution B.
8. After maintaining for 1 day, aspirate Solution B and switch back to Solution A for induction.
9. Alternate between Solution A (3 days) and Solution B (1 day). Monitor cell morphology daily. If cells show signs of shrinkage or detachment during Solution A treatment, switch to Solution B immediately until the cell state stabilizes.

Note:

1) **Solution A stimulates lipid droplet formation, while Solution B maintains and promotes the enlargement of these droplets.**
2) **Typically, efficient adipogenesis is achieved using a cyclic induction of "3 days in Solution A followed by 1 day in Solution B".**
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 cycles until sufficient lipid droplets of appropriate size have formed, indicating the cells are ready for staining.

Oil Red O Staining Analysis

Materials Required

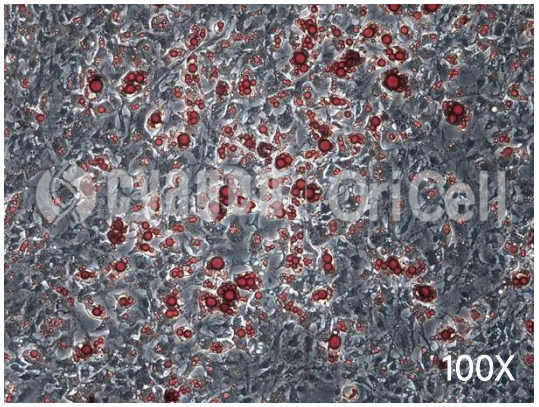
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- 4% Paraformaldehyde Solution or 10% Formalin Solution
- OriCell™ Oil Red O Solultion (Cat. No.: OILR-10001)

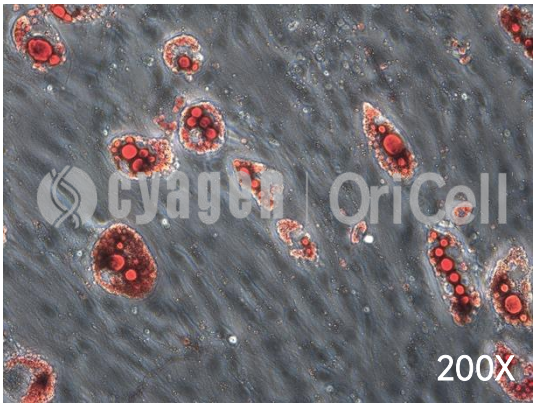
Steps

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

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. Prepare the Oil Red O working solution by mixing OriCell™ Oil Red O Solution (Cat. No.: OILR-10001) with 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 and gently wash 2–3 times with 1× PBS to ensure complete removal.
5. Add 2 mL of Oil Red O working solution to each well and incubate for 30 minutes at room temperature.
6. Aspirate the solution and rinse the wells 2–3 times with 1× PBS to ensure thorough removal of excess stain.
7. Add 2 mL of 1× PBS to each well and observe the staining results under a microscope.
8. Seal the plate with Parafilm and protect from light. The stained plate can be stored at 4 °C for up to 1 week. Prolonged storage may cause lipid droplets to fuse, altering the original stained morphology.

Oil Red O Staining Results





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