cyagen | OriCell™

User Manual

OriCell™ Chondrogenic Differentiation Medium For Rat Adipose-derived Mesenchymal Stem Cells

Catalog No. RAXMD-90041

We help you discover life

Introduction

OriCell™ Chondrogenic Differentiation Medium For Rat Adipose-derived Mesenchymal Stem Cells, developed by the R&D team, contains a basic medium and supplements suitable for the growth of rat adipose-derived mesenchymal stem cells. Extensive cell culture data have demonstrated that this product can induce these cells to differentiate into cartilage tissue stably and efficiently.

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	BHDM-03011	97 mL
OriCell™ Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation I	RAXMD-04041-1	2 mL
OriCell™ Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation II	RAXMD-04041-2	1 mL
OriCell™ Alcian Blue 8GX Solultion (pH=2.1~2.3)	ALCB-10001	5 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 the complete medium cannot be used up within a short period, prepare it in batches according to the volume ratio of each component in the kit, and store in aliquots for future use.

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 up to 1 month. If culture conditions remain stable, the container is well-sealed, and temperature fluctuations are avoided, the storage period may be extended appropriately but should not exceed 45 days.
4. Please use all products before the expiration date. Expired ingredients may significantly affect the cell culture effect.

Preparation of Premix Solution

Materials Required

- OriCell™ Chondrogenic Differentiation Medium For Rat Adipose-derived Mesenchymal Stem Cells (Cat. No.: RAXMD-90041)
- 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 30 minutes before preparation, place OriCell™ Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation I (Cat. No.: RAXMD-04041-1) in a refrigerator at 4°C until it is completely thawed.
2. Gently invert or flick the reagent tube to mix the contents thoroughly.
3. Carefully wipe the outer packaging of all ingredients with 75% ethanol. Open the package inside a clean bench.
4. Add Supplement I (Cat. No.: RAXMD-04041-1) to OriCell™ Basal Medium For Cell Culture (Cat. No.: BHDM-03011).

5. Use a small amount of basal medium to rinse each bottle and tube, then transfer all ingredients into the basal medium to maximize ingredient recovery.
6. Tighten the cap of the basal medium bottle and shake gently and thoroughly to mix.
7. Seal the bottle with parafilm, wrap it with aluminum foil, and label it with the name, preparation date, and other relevant information.

Special Reminder

- If the medium will not be used up immediately, we recommend preparing in batches. Please prepare required volume 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™ Chondrogenic Differentiation Medium For Rat Adipose-derived Mesenchymal 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 complete medium can be filtered and sterilized.
- The prepared chondrogenic differentiation medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Chondrogenic Differentiation Medium For Rat Adipose-derived Mesenchymal Stem Cells (Cat. No.: RAXMD-90041)
- 4% Paraformaldehyde solution Or 10% Formalin solution

Steps

1. Transfer 3–4 × 10^5 cells to a 15 mL centrifuge tube and centrifuge at 250 × g for 4 minutes at 20°C.
2. Aspirate the supernatant. Resuspend the cell pellet from the previous centrifugation in 0.5 mL chondrogenic differentiation premix, then centrifuge at 150 × g for 5 minutes at 20°C.
3. Repeat the centrifugation and resuspension as described in step 2.
4. Add OriCell ™ Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation II (Cat. No.: RAXMD-04041-2) to the chondrogenic differentiation premix at a ratio of 10 μL per 1 mL. Mix the required volumes thoroughly by gentle pipetting to prepare the complete chondrogenic differentiation medium.

   Note:

   1) Briefly centrifuge the reagent tube before pipetting Supplement II (Cat. No.: RAXMD-04041-2), to collect the reagent at the bottom of the tube.

   2) Store Supplement II in aliquots at -20 °C or below. Avoid repeated freezing and thawing. The reagent can be stored for up to 12 months.

   3) The complete chondrogenic differentiation medium containing Supplement II should be prepared fresh and used immediately, or stored at 4°C for no longer than 12 hours.

5. Resuspend the cell pellet from step 3 in 0.5 mL of complete chondrogenic differentiation medium, then centrifuge at 150 × g for 5 minutes at 20°C.
6. Loosen the centrifuge tube cap to allow gas exchange, and place the tube upright in a CO_2 incubator at 37°C with 5% CO_2 and saturated humidity.

   Note:

   1) Aspirating the supernatant or resuspending the cells is not required in this step.

   2) Do not shake or agitate the centrifuge tube for 24 hours.

7. When cell clusters form (usually after 24–48 hours, depending on the conditions), gently flick the bottom of the centrifuge tube to detach the cartilage ball and suspend it in the medium.
8. Starting from the time of inoculation, replace the medium every 2–3 days with fresh complete chondrogenic differentiation medium, approximately 0.5 mL per tube.

Note:

1) Be careful not to aspirate the cartilage ball.

2) After changing the medium, gently flick the bottom of the centrifuge tube to detach the cartilage ball and suspend it in the medium.

3) After each medium change, be sure to loosen the tube cap and place the tube in the incubator.

9. Continue the induction process until a cartilage ball with a diameter of 1.5–2 mm forms in the tube, after which sections can be prepared for staining.

Cartilage Ball Section Staining

Materials Required

- Induced cartilage balls
- 4% Paraformaldehyde Solution or 10% Formalin Solution
- Anhydrous Alcohol, Xylene, Paraffin Wax
- OriCell™ Alcian Blue 8GX Solution (Cat. No.: ALCB-10001)

Steps

Note: **This protocol uses paraffin section staining as an example. If frozen sections are used, please follow the corresponding microtome instructions.**

1. Fixation: Wash the induced cartilage balls with 1× PBS, then immerse them in 4% paraformaldehyde (or 10% formalin) solution and fix for at least 30 minutes.

   Note: **The fixative should be freshly prepared and used immediately.**

2. Dehydration: Dehydrate the fixed cartilage balls through a graded series of ethanol, with each step lasting 30 minutes, as follows:

   50% ethanol → 70% ethanol → 80% ethanol → 95% ethanol → Anhydrous ethanol

Note:

1) Perform dehydration in a covered container to prevent moisture absorption from the air.

2) Do not soak the cartilage balls in ethanol for too long, as they are prone to fragmentation.

3) When replacing ethanol, carefully remove the lower concentration ethanol before adding the higher concentration solution. Avoid excessive movement to reduce the risk of cartilage ball breakage.

3. Transparency. Since ethanol and paraffin wax are immiscible, the samples must be treated with xylene after dehydration. Proceed as follows:

① Mix xylene and anhydrous ethanol at a 1:1 volume ratio. Soak the cartilage balls in the mixture for 2 hours.

② Soak the cartilage balls in pure xylene for 1.5 hours.

③ Replace with fresh xylene and continue soaking for 1 hour.

Note:

1) Perform the transparency step in a covered container to prevent moisture absorption from the air.

2) If white misty bubbles appear around the cartilage balls during the transparency process, it indicates incomplete dehydration. Return the samples to ethanol for further dehydration before continuing transparency.

4. Dip wax (Wax infiltration). To remove the clearing agent from the cartilage balls and allow paraffin to penetrate for embedding, perform wax treatment as follows:

① Mix xylene and paraffin at a volume ratio of 1:1. Soak the cartilage balls in this mixture and incubate in an oven at 40°C for 40 minutes.

② Transfer the cartilage balls to pure paraffin and incubate in an oven at 55°C for 30 minutes.

Note:

1) Keep the wax immersion temperature as low as possible to prevent the paraffin wax from solidifying.

2) The temperature must be kept constant without fluctuations.

5. Embedding. Remove the cartilage balls and place them into molds. Pour in paraffin and allow it to cool and solidify completely. Once fully set, remove the paraffin block and trim as needed.
6. Sectioning. Cut continuous sections at a thickness of 3 μm.
7. Mounting and drying. Adhere the cartilage sections onto glass slides using an adhesive, then dry them in an oven at 35°C.
8. Dewaxing.

   ① Soak the sections in pure xylene for 15 minutes, then replace with fresh xylene and soak for another 10 minutes.

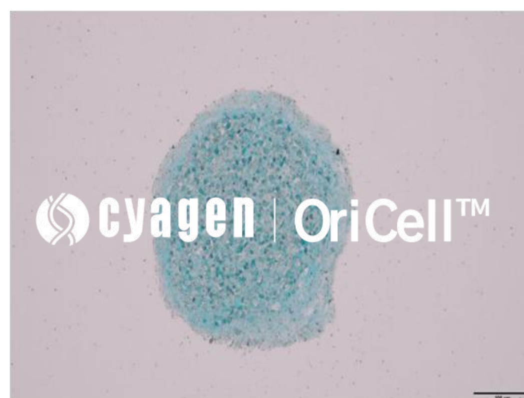
   ② Soak the sections in a 1:1 mixture of xylene and anhydrous ethanol for 10 minutes.

   ③ Sequentially soak the sections in 95%, 85%, 70%, and 50% ethanol for 10 minutes each, then air dry.
9. Staining

   ① Apply OriCell™ Alcian Blue 8GX Solution dropwise onto the dried sections and incubate at 37°C for 1 hour.

   ② Rinse the slides under tap water for 5 minutes and air dry.
10. Observation. Examine the staining under a microscope. Areas stained with OriCell™ Alcian Blue 8GX Solution indicate the presence of acidic mucopolysaccharides within the cartilage tissue.

The Alcian Blue 8GX Staining Effect



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