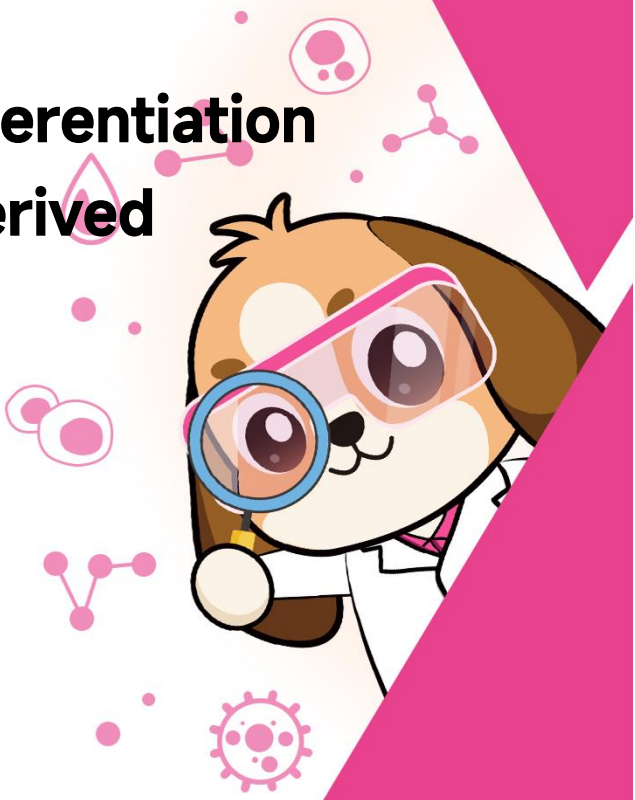
cyagen | OriCell™

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
Alcian Blue 8GX Solution (pH=2.2)	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 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 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 within 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. Invert or flick the reagent tube to mix the reagent thoroughly.
3. Carefully wipe the outer packaging of all components with 75% ethanol. Open the packages 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. 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.
6. Tighten the cap of the basal medium bottle and shake it gently and thoroughly.
7. 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 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™ 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 be induced to a 15 mL centrifuge tube at 20°C. Centrifuge at 250×g for 4 min.
2. Aspirate the supernatant. Add 0.5 mL chondrogenic differentiation premix solution, resuspend the pellet obtained from the previous centrifugation, and centrifuge at 150×g for 5 min at 20°C.
3. Repeat step 2, wash the cells again.
4. Add OriCell™ Supplement For Rat Adipose-derived Mesenchymal Stem Cells Chondrogenic Differentiation II (Cat. No.: RAXMD-04041-2) according to the ratio (1 mL of Chondrogenic Differentiation Premix, add 10 μ L Cat. No. RAXMD-04041-2), absorb the required dose of Supplement II for the experiment, add it into a corresponding volume of the premix to mix into the chondrogenic differentiation medium, gently pipette to mix.

   Note:

   1) Centrifuge the reagent tube briefly before sucking supplement II (Cat. No.: RAXMD-04041-2), so that the reagent can be collected at the bottom of the tube for use.

   2) Be sure to store supplement II (Cat. No.: RAXMD-04041-2) in aliquots below -20 °C, avoid repeated freezing and thawing, and can be stored for 12 months.

   3) The chondrogenic differentiation medium added with supplement II (Cat. No.: RAXMD-04041-2) must be prepared for immediate use, and stored at 4°C for no more than 12 h.
5. Resuspend the cell pellet obtained in step 3 in 0.5 mL of chondrogenic differentiation medium, and centrifuge at 150×g for 5 min at 20°C.
6. Unscrew the cap of the centrifuge tube to facilitate gas exchange. Place it upright in a CO_2 incubator at 37°C, 5% CO_2, and saturated humidity.

   Note:

   1) This step does not need to aspirate the supernatant or resuspend the cells.

   2) Do not shake the centrifuge tube within 24 hours.
7. When the cell clusters appear agglomerated (usually after 24 h or 48 h, depending on the actual situation), slightly flick the bottom of the centrifuge tube to make the cartilage ball detach from the

bottom of the tube and suspend it in the liquid.

Calculated from the beginning of inoculation, replace the cells with fresh chondrogenic differentiation medium every 2 to 3 days, about 0.5 mL per tube.

Note:

1) Be careful not to suck out the cartilage ball.

2) After each medium change, you need to flick the centrifuge tube to make the cartilage ball detach from the bottom of the tube and suspend in the liquid.

3) After changing the medium each time, be sure to loosen the cap of the centrifuge tube and put it into the incubator.

8. Continue to induce until a cartilage ball with a diameter of 1.5-2 mm is formed in the tube, and the section 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 step takes paraffin section staining as an example. If using frozen sections, please follow the instructions for using the microtome.**

1. Fixed. The induced cartilage balls were washed with 1 × PBS, soaked in 4% paraformaldehyde solution (or 10% formalin solution), and fixed for more than 30 minutes.

Note: **The fixative solution needs to be prepared for immediate use.**

2. Dehydration. The fixed cartilage balls are dehydrated with gradient concentration of alcohol in the following way, each stage is 30 minutes.

50% alcohol ⟶ 70% alcohol ⟶ 80% alcohol ⟶ 95% alcohol ⟶ Anhydrous alcohol

Note: **1) Dehydration must be carried out in a covered container to prevent absorbing moisture in the air.**

2) Do not soak the cartilage balls in alcohol for too long, as they are easily broken.

3) When changing alcohol, suck out low-concentration alcohol, and then add high-concentration alcohol. Avoiding movement increases the risk of fragmentation of the cartilage ball.

3. Transparency. Because alcohol and paraffin wax are incompatible, it needs to undergo xylene transition after dehydration. The way is as follows:

① Mix xylene and absolute alcohol at a volume ratio of 1:1. Soak the cartilage ball in it for 2 hours.

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

③ Replace with new xylene and continue to soak for 1 hour.

Note: **1) Transparency must be carried out in a covered container to prevent absorbing moisture in the air.**

2) During the transparent process, if white misty bubbles appear around the cartilage ball, it means that the water has not been removed. It should be put back in the alcohol for dehydration and then transparent.

4. Dip wax. In order to remove the transparent agent in the cartilage ball and make the paraffin penetrate into the interior for embedding, a wax immersion treatment is required.

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

② Soak the cartilage balls in pure paraffin and place them in an oven at 55°C for 30 minutes.

Note:

1) The wax immersion should be kept at a lower temperature as much as possible, so that the paraffin wax does not solidify.

2) The temperature must be constant.

5. Embedding. Take out the cartilage ball and place it in the mold. Pour in paraffin wax and let it stand to cool. After the paraffin is fully cooled and formed, take it out
6. Slicing. Continuous slices, each with a thickness of 3 μm.
7. Sticky film. The cartilage ball slices were adhered to the glass slide with an adhesive, and dried in an oven at 35°C.
8. Dewaxing.
   ① Soak the slices in pure xylene for 15 minutes, and replace the slices with new xylene for 10 minutes.
   ② Xylene and anhydrous alcohol are mixed in a volume ratio of 1:1. Soak the slices for 10 minutes.
   ③ Use 95%, 85%, 70%, and 50% alcohol to soak the slices for 10 minutes, and then dry them.
9. Dyeing.
   ① Add alicin blue dropwise to the dried section and stain at 37°C for 1 h.
   ② Rinse the slides with tap water for 5 minutes and dry them.
10. Observe the staining effect of Alcian Blue under the microscope. Alcian blue staining partially shows the internal acid mucopolysaccharide in cartilage tissue.

The Alcian Blue 8GX Staining Effect