cyagen | OriCell

User Manual

OriCell™ Chondrogenic Differentiation Medium For ATDC-5 Cells

Catalog No. CLATD-90041

We help you discover life

Introduction

OriCell™ Chondrogenic Differentiation Medium For ATDC-5 Cells is specially formulated and developed by our R&D team. It consists of an optimized basal medium and essential supplements for the induction of chondrogenic differentiation in ATDC-5 cells.

Extensive cell culture data have demonstrated that this medium supports the stable and efficient differentiation of ATDC-5 cells into chondrocytes.

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	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture	BHDM-03011	97 mL	4 °C
OriCell™ Supplement For ATDC-5 Cells Chondrogenic Differentiation I	CLATD-04041-1	2 mL	-20 °C
OriCell™ Supplement For ATDC-5 Cells Chondrogenic Differentiation II	CLATD-04041-2	1 mL	-20 °C
Alcian Blue 8GX Solution (pH=2.2)	ALCB-10001	5 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 has 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™ Chondrogenic Differentiation Medium For ATDC-5 Cells (Cat. No.: CLATD-90041)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil and other light-avoiding materials

Steps

Note: The final mixture prepared in this section is referred to as "premixed solution".

1. At least 30 minutes before preparation, place the OriCell ™ Supplement For ATDC-5 Cells Chondrogenic Differentiation I (Cat. No.: CLATD-04041-1) in a refrigerator at 4 °C to allow it to

thaw completely.

2. Mix the reagents by inverting the tube several times or gently flicking the bottom of the tube.
3. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
4. Add all of the Supplement I (Cat. No.: CLATD-04041-1) to OriCell™ Basal Medium (Cat. No.: BHDM-03011).
5. Rinse the Supplement I tube with a small volume of basal medium, then transfer the washings back to the basal medium bottle to maximize recovery.
6. Securely tighten the cap on the basal medium bottle. Mix thoroughly by gentle swirling or inversion.
7. 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™ Chondrogenic Differentiation Medium For ATDC-5 Cells are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilizing it again. However, if there is a risk of contamination during the preparation process, the complete 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™ Chondrogenic Differentiation Medium For ATDC-5 Cells (Cat. No.: CLATD-90041)
- 4% Paraformaldehyde Solution or 10% Formalin Solution

Steps

1. Transfer 3–4 x 10^5 cells into a 15 mL centrifuge tube and centrifuge at 250 × g for 4 minutes at 20 °C.
2. Aspirate the supernatant. Resuspend the cell pellet in 0.5 mL premixed solution (prepared above), then centrifuge at 150 × g for 5 minutes at 20 °C.
3. Repeat the wash step once more as described in Step 2.
4. Add OriCell™ Supplement For ATDC-5 Cells Chondrogenic Differentiation II (Cat. No.: CLATD-04041-2) to the premixed solution at a 1:100 ratio (e.g., 10 µL Supplement II per 1 mL premixed solution). Mix thoroughly by gentle pipetting to prepare the complete chondrogenic differentiation medium (i.e., working induction medium).

   Notes:

   1) **Briefly centrifuge the reagent tube before pipetting Supplement II 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 working induction medium (containing Supplement II) should be prepared fresh and used immediately, or stored at 4 °C for no longer than 12 hours.**

5. Aspirate the supernatant from Step 3, resuspend the cell pellet in 0.5 ~ 1 mL of working induction 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) Do not aspirate the supernatant or resuspend the cells in this step.

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

7. Once the cells have aggregated into a visible pellet (typically after 24–48 hours, depending on cell state), gently flick the bottom of the centrifuge tube to detach the pellet from the surface, allowing it to suspend in the medium.
8. Starting from the time of seeding, replace the medium every 2–3 days with fresh working induction medium (approximately 0.5 ~ 1 mL per tube).

Note:

1) Be careful not to aspirate the cartilage spheroid.

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

3) Ensure the tube cap is loosened prior to returning the tube to the incubator.

9. Continue the induction process until a cartilage spheroid with a diameter of 1.5–2 mm forms in the tube, at which point it is ready for sectioning and staining.

Cartilage Spheroid Sectioning and Staining

Materials Required

- Induced cartilage spheroids
- 4% Paraformaldehyde solution or 10% Formalin solution
- Absolute ethanol, xylene, paraffin (or Paraffin wax)

- OriCell™ Alcian Blue 8GX Solultion (Cat. No.: ALCB-10001)

Steps

Note: **This protocol uses paraffin section staining as an example. If frozen sections are used, sectioning procedures may differ.**

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

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

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

   50% Ethanol → 70% Ethanol → 80% Ethanol → 95% Ethanol → Absolute Ethanol

   (30 minutes each)

   Note:

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

   2) Do not soak the cartilage spheroids 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 spheroid breakage.

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

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

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

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

   Note:

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

   2) If white misty bubbles appear around the cartilage spheroids during the clearing process, it

indicates incomplete dehydration. Return the samples to ethanol for further dehydration before continuing clearing.

4. Wax Infiltration. To remove the clearing agent from the cartilage spheroids 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 spheroids in this mixture and incubate in an oven at 40 °C for 40 minutes.
   ② Transfer the cartilage spheroids to pure paraffin and incubate in an oven at 55 °C for 30 minutes.

   Note:

   1) Keep the wax immersion temperature high enough and stable to ensure that the paraffin remains fully molten. Avoid excessive heat to prevent tissue damage.

   2) The temperature must be kept constant without fluctuations.

5. Embedding. Remove the cartilage spheroids and place them into molds. Pour in paraffin and allow them to cool and solidify completely. Once the paraffin block has completely solidified, remove it and trim as needed.
6. Sectioning. Cut continuous sections at a thickness of 3 μm.
7. Mounting and drying. Mount the 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 absolute 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 Alcian Blue indicate the presence of acidic mucopolysaccharides within the cartilage matrix.

The Alcian Blue Staining Result