cyagen | OriCell

User Manual

OriCell™ Osteoclast Differentiation Medium For RAW264.7 (Pro)

Catalog No. CLROP-90401

We help you discover life

Introduction

OriCell™ Osteoclast Differentiation Medium For RAW264.7 (Pro) is meticulously developed by our R&D team for the differentiation of RAW 264.7 cells into osteoclasts. The kit consists of a specialized differentiation medium, essential supplements and staining solutions, providing a complete solution for osteoclast differentiation.

Extensive validation studies have demonstrated that this product can stably and efficiently induce the differentiation of RAW 264.7 cells into osteoclasts in vitro. The optimized formulation supports reproducible differentiation outcomes and consistent osteoclast formation, facilitating reliable downstream applications in bone biology, osteoimmunology, and drug discovery research.

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 Medium Kit	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture	BAME-03011	45 mL	4 °C
OriCell™ Supplements For Osteoclast Differentiation I	CLROP-04401	5 mL	-20 °C
OriCell™ Supplements For Osteoclast Differentiation II	CLROP-04402	25 μL	-20 °C
OriCell™ Supplements For Osteoclast Differentiation III	CLROP-04403	12.5 μL	-20 °C

Components of Staining Solutions	Catalog Number	Volume	Storage
OriCell™ TRAP Staining Solution Component I	TRAP-10001	1 mL	-20 °C
OriCell™ TRAP Staining Solution Component II	TRAP-10002	100 μL	-20 °C
OriCell™ TRAP Staining Solution Component III	TRAP-10003	9 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. All supplements have a shelf life of 1 year and should be stored at −20 °C. The staining solutions have a shelf life of 6 months and should be stored at 4 °C (III) and -20 °C (I & II) separately.
3. Once prepared, the medium has a shelf life of 1 month when stored at 4 °C.
4. Use all components before the expiration dates. Expired components may severely compromise culture performance.

Preparation of Complete Medium

Materials Required

- OriCell™ Osteoclast Differentiation Medium For RAW264.7 (Pro) (Cat. No.: CLROP-90401)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil and other light-avoiding materials

Steps

1. At least 3 hours before preparation, place all supplements (Cat. No.: CLROP-04401, CLROP-04402, CLROP-04403) in a refrigerator at 4 °C to allow them to thaw completely.
2. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package inside a clean bench (laminar flow hood).
3. Add all supplements (Cat. No.: CLROP-04401, CLROP-04402, CLROP-04403) to OriCell™ Basal Medium (Cat. No.: BAME-03011).
4. Tighten the cap of the basal medium bottle and swirl the bottle gently to mix thoroughly.
5. 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.

Note:

1) 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.

2) All components in the OriCell™ Osteoclast Differentiation Medium For RAW264.7 (Pro) 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.

3) The prepared medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Osteoclast Differentiation Medium For RAW264.7 (Pro) (Cat. No.: CLROP-90401)
- OriCell™ RAW 264.7 Mouse Monocyte Macrophage Leukemia Cell Line (Cat. No.: M3-0101)
- OriCell™ Complete Medium For RAW 264.7 Cell Line (Cat. No.: CMM3-0101)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)

Steps

Note:

1) This protocol uses a 12-well plate as an example. Please select an appropriate culture vessel based on your specific needs.

2) Pre-warm the induction medium to 37 °C before use.

3) This experiment uses RAW 264.7 cells as an example.

1. Seed RAW 264.7 cells into a 12-well plate at a density of 1×10^4 cells per well, and add 1 mL of complete medium (Cat. No.: CMM3-0101) to each well.
2. Incubate the cells in a humidified incubator at 37 °C with 5% CO_2.
3. After 48 hours, carefully aspirate the complete medium from each well and replace it with 1

mL of differentiation medium (Cat. No.: CLROP-90401).

4. Perform a medium change every other day using fresh differentiation medium.
5. After 4–7 days of induction, perform TRAP staining using the TRAP staining solutions based on cell morphology and growth status.

TRAP Staining Protocol

Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- 4% Paraformaldehyde solution or 10% Formalin solution
- OriCell™ TRAP Staining Solutions (Cat. No.: TRAP-10001, TRAP-10002, TRAP-10003)

Steps

Note:

1) To prevent cell detachment, perform all procedures as gently as possible.

2) If staining intensity is weak, the staining time may be appropriately extended.

3) Ensure that osteoclasts have formed before performing TRAP staining.

4) The staining solution should be freshly prepared before use.

1. After completion of RAW 264.7 osteoclast differentiation, aspirate the differentiation medium from the 12-well plate and gently wash the cells 1 – 2 times with 1× PBS.
2. Add 1 mL of 4% paraformaldehyde solution (or 10% formalin) to each well and fix at room temperature for 10 – 30 minutes.
3. Aspirate the fixative and gently wash the cells 1 – 2 times with 1× PBS to ensure complete removal of the fixative.
4. Prepare the TRAP working solution by mixing component I (Cat. No.: TRAP-10001), II (Cat. No.:

TRAP-10002), and III (Cat. No.: TRAP-10003) at a ratio of **10 : 1 : 90**.

5. Add approximately 1 mL of TRAP working solution to each well and incubate in a humidified incubator at 37 °C with 5% CO_2 for 1 – 2 hours.
6. Aspirate the TRAP staining solution and gently wash the cells 2 – 3 times with 1× PBS to remove excess stain.
7. Add 1 mL of 1× PBS to each well and observe the staining results under a microscope (see the following image for expected staining results).
8. Seal the stained 12-well plate with Parafilm. The plate can be stored at 4 °C for up to 2 weeks.

TRAP Staining Example

TRAP Staining of Osteoclasts Induced from RAW 264.7 Cells