ATCC

TECHNICAL NOTE

OPTIMIZED PROTOCOL FOR ATCC ASSAY READY THP-1 MONOCYTE DIFFERENTIATION WITH PMA

INTRODUCTION:

This protocol provides instructions for differentiating ATCC Assay Ready THP-1 monocytes (ATCC® TIB-202-AR™) into macrophage-like cells using phorbol 12-myristate-13-acetate (PMA). ATCC Assay Ready THP-1 monocytes eliminate the need for any prior cell culturing and are directly plated in this assay from the frozen state—simply thaw and go!

PMA is a potent activator of Protein Kinase C, which in turn activates NF-κB in vitro. Although PMA is a commonly used agent for in vitro macrophage differentiation, the conditions used (PMA concentration, length of treatment, etc.) vary widely from lab to lab. The lack of a standardized protocol has resulted in THP-1–derived macrophage populations that are inconsistent and differ significantly in terms of phenotype and function. Here, we provide an optimized protocol that can be used to differentiate ATCC Assay Ready THP-1 monocytes into macrophage-like cells with high efficiency and consistency.

GENERAL CONSIDERATIONS:

- All steps should be performed in a biosafety cabinet using proper aseptic technique.
- **Assay Ready Cells should be thawed using the recommended thawing procedure for Assay ready cells available on the ATCC website and product sheet.**
- **Assay Ready Cells can be seeded immediately post-thaw.**
- The general suggestions below have been demonstrated to yield macrophage-like cells consistently; for best results, the differentiation conditions may need to be optimized for each specific application/assay.

MATERIALS REQUIRED:

Material required	Catalog No.
Vial of Assay Ready THP-1 cells	ATCC® TIB-202-AR™
RPMI	ATCC® 30-2001™
FBS (10%)	ATCC® 20-2020™
2-Mercaptoethanol (0.05 mM)	
DMSO	ATCC® 4-X™
PMA	Sigma™, P185-10MG
Optional: cell scraper or Trypsin	ATCC® 30-2101™

PREPARATION:

1. **Complete Media Preparation:**
   - Use freshly prepared media containing :
     - RPMI
     - 10% FBS
     - 0.05 mM 2-mercaptoethanol
   - Filter sterilize the media (0.22 µm cellulose acetate membrane, or similar).
2. **PMA preparation:**
   - Dilute PMA to a stock solution of 0.5 mg/mL with DMSO (ATCC 4-X). Filter sterilize.
   - Aliquot and freeze. Ensure to avoid light exposure as PMA is sensitive and avoid repeated freeze/thaw.
3. **Differentiation Media Preparation:**
   - Add PMA to the complete media at a working final concentration of 100 ng/mL for this assay

CELL SEEDING AND DIFFERENTIATION PROTOCOL:

4. **Seed cells:**
   - After thawing the Assay Ready Cells (follow ATCC recommended thawing procedure for Assay Ready Cells available on the ATCC website and product sheet), seed the cells at a density of 600,000 cells/mL to multi-well culture dish.
5. **Cell distribution:**
   - Move plates/dishes up and down and side to side to evenly distribute cells (check under a microscope).
6. **Incubation:**
   - Incubate the cells at 37°C with 5% CO_2.
7. **Monitoring:**
   - After 24 hours:
     - Check the cells under a microscope. Cells treated with PMA will adhere to the dish and start changing morphology.
     - Return cells to the incubator.
   - After 48 hours:
     - Check the cells under a microscope. Cells will continue adhering to the dish and changing morphology.
     - Replace the media by aspirating old media and replacing it with fresh media containing 100 ng/mL PMA.
     - Return to the incubator.
8. **Assay preparation:**
   - After 72 hours, cells may be imaged and then fixed and/or harvested for assay.
   - Cells should be strongly adhered to the dish, and a majority will exhibit a macrophage-specific morphology (larger cytoplasmic volume and increased granularity).
   - Immunocytochemistry and other imaging-based assays can be conducted directly on plated cells.
   - For reference images and application data, refer to the ATCC product page.
9. **Cell detachment (if needed):**
   - Cells can be detached using a cell scraper or trypsin for use in other assays.

For more information visit www.atcc.org

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