ATCC

# TECHNICAL NOTE

ThawReady™ by ATCC

# Optimized protocol for ATCC ThawReady™ THP-1 monocyte differentiation with PMA

## INTRODUCTION:

This protocol provides instructions for differentiating ATCC ThawReady™ THP-1 monocytes (ATCC® TIB-202-AR™) into macrophage-like cells using phorbol 12-myristate-13-acetate (PMA). ATCC ThawReady™ 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 ThawReady™ 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.
- **ThawReady™ Cells should be thawed using the recommended thawing procedure for ThawReady™ cells available on the ATCC website and product sheet.**
- **ThawReady™ 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 ThawReady™ 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 ThawReady™ Cells (follow ATCC recommended thawing procedure for ThawReady™ 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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