ThawReady™ by ATCC

# Development of a ThawReady™ THP-1 Product for Cell-Based Assays

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## ABSTRACT

Cell-based assays are extensively used in both research and industry for various applications, including target identification, drug development, and compound toxicity testing. A major challenge with cell-based assays is the inherent variability of cultured cells. Contributing factors include cell culture practices, phenotypic drift associated with long-term cultivation, and biomaterials sourced from different laboratories. The disadvantages of maintaining continuous cultures have driven the demand for cell products that are ready for immediate use in assays. To meet this need, ATCC has developed an assay-ready cell (ARC) product using the THP-1 cell line: ThawReady™ THP-1 (ATCC® TIB-202-AR™). In the following study, we will showcase the development of this novel product and demonstrate that it consistently exhibits high post-thaw viability and can differentiate into macrophage-like cells that express the appropriate markers and display expected functional attributes.

## INTRODUCTION

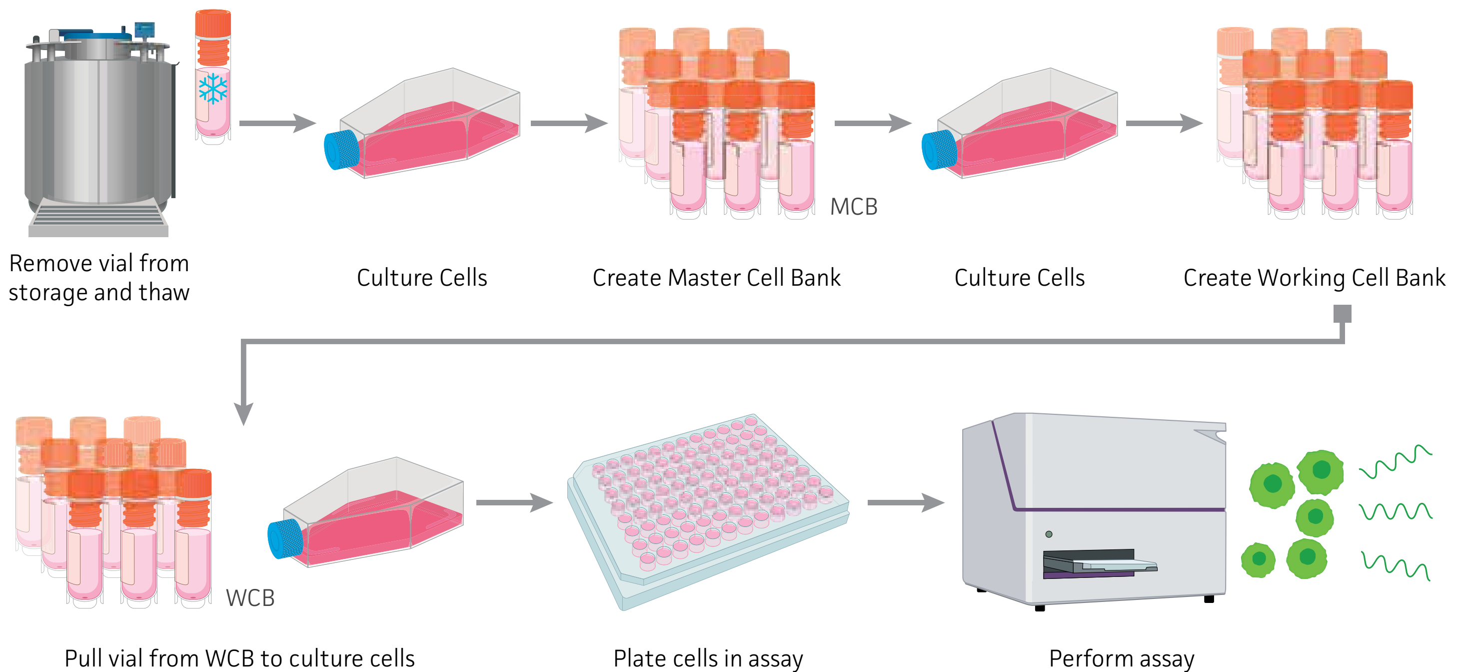
Cell-based assays are valuable tools for basic research and drug discovery. Traditionally, these assays involve thawing frozen cells and culturing them through multiple passages to reach the required density for assay. However, in continuous culture, most cells do not maintain their characteristics indefinitely. As the passage number increases over time, cells may acquire unintended or unexpected characteristics, and their responsiveness to certain modulators can change—a phenomenon known as phenotypic drift.[1] Phenotypic drift presents one of the greatest challenges in achieving consistent, high-performance cell-based in vitro assays. Additionally, traditional cell-based assays have several disadvantages, including the labor required to maintain cell culture, the cost of consumables such as media and laboratory plasticware, the need for laboratory space, and the use of dedicated equipment like incubators.

Assay ready cells (ARCs) represent a significant advancement in cell-based assay applications. ARCs can be used directly in assays without the need for prior cell cultivation, reducing the potential for variability that may arise (Figure 1). To develop high-quality ARCs, it is essential to establish and validate good cell culture practices to ensure consistent cell performance. Optimized cryopreservation media and cell freezing protocols are also crucial for preparing ARCs. While various tests can be utilized to evaluate ARC performance, post-thaw viability and functional performance should always be measured to provide critical information about the quality of the ARCs.

THP-1 (ATCC® TIB-202™) is a human monocytic cell line derived from the blood of a patient with acute monocytic leukemia. It is one of

the most widely used and physiologically relevant cell models, though it can be challenging to culture.[2] Leveraging our proprietary animal by-product (ABP)-free cryopreservation medium and well-established high-standard cell culture practices, ATCC has developed a THP-1 ARC product: ThawReady™ THP-1 (ATCC® TIB-202-AR™). This product is manufactured to meet defined specifications for low intra-lot and inter-lot variation and demonstrates reproducibility in achieving optimal performance in cell-based assays. ThawReady™ THP-1 cells consistently exhibit high post-thaw viability and expected functional attributes, offering several advantages for establishing cell-based assays, including long-term access to a consistent resource, more flexible scheduling, and cost savings.

### THE TRADITIONAL CELL CULTURE WORKFLOW




### THE THAWREADY™ WORKFLOW

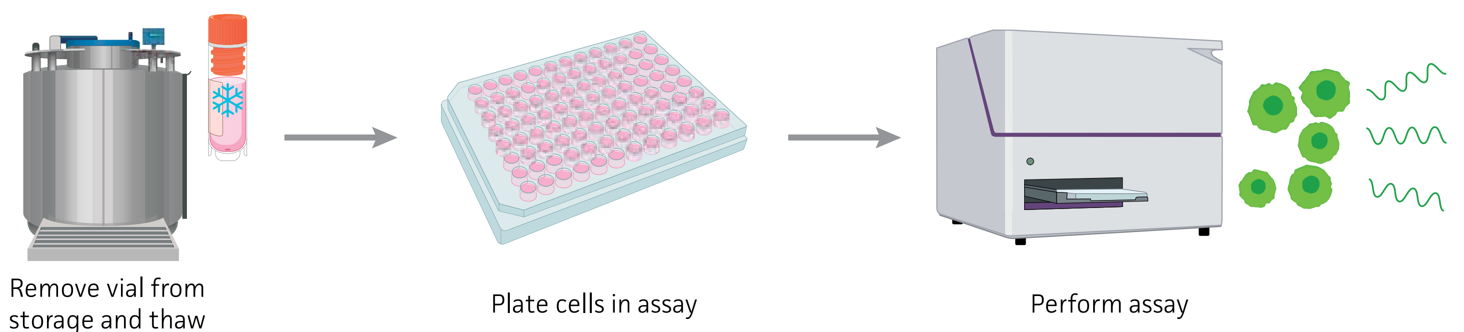



**Figure 1: Overview of the traditional cell-based assay and ARC-based assay workflows.** Traditional cell-based assays have lengthy timelines due to the requirement of cell expansion processes to get a synchronized cell stock. To speed your timelines while providing you with the consistency you need, ATCC developed a new ARC product. ARCs are ready within hours of thawing and are scalable for high-throughput assays, thereby eliminating lengthy cell expansion processes and streamlining your workflow by months.

## RESULTS WITH MATERIALS AND METHODS

To establish the cell expansion process for developing ThawReady™ THP-1 cells, we grew parental THP-1 cells (ATCC® TIB-202™) in RPMI-1640 (ATCC® 30-2001™) supplemented with 10% Fetal Bovine Serum (ATCC® 30-2020™) and 0.9 µL/mL of 2-Mercaptoethanol (Thermo Fisher Scientific® #21985-023) following ATCC cell culture protocols. Cells were monitored for density and sub-cultured when they reached the desired confluency levels. During the cell expansion process, culture conditions (e.g., seeding density, cell viability, viable cell counts, and culture length between sub-cultures) were closely monitored and recorded to ensure consistency across different batches handled by various operators.

THP-1 cells were frozen following an optimized cryopreservation protocol with ATCC'S carefully formulated and proprietary ABP-free cryopreservation media using a CryoMed™ Controlled-Rate Freezer (Thermo Fisher Scientific®). The manufactured ThawReady™ THP-1 cells were deposited in the vapor phase of liquid nitrogen (LN2) for long-term storage. ThawReady™ THP-1 cells from at least three different batches were thawed by various operators, and post-thaw viability was measured using a Vi-CELL BLU® cell viability analyzer (Beckman Coulter®). The ThawReady™ THP-1 cells consistently exhibited high post-thaw viability with low intra-lot and inter-lot variation (Figure 2).

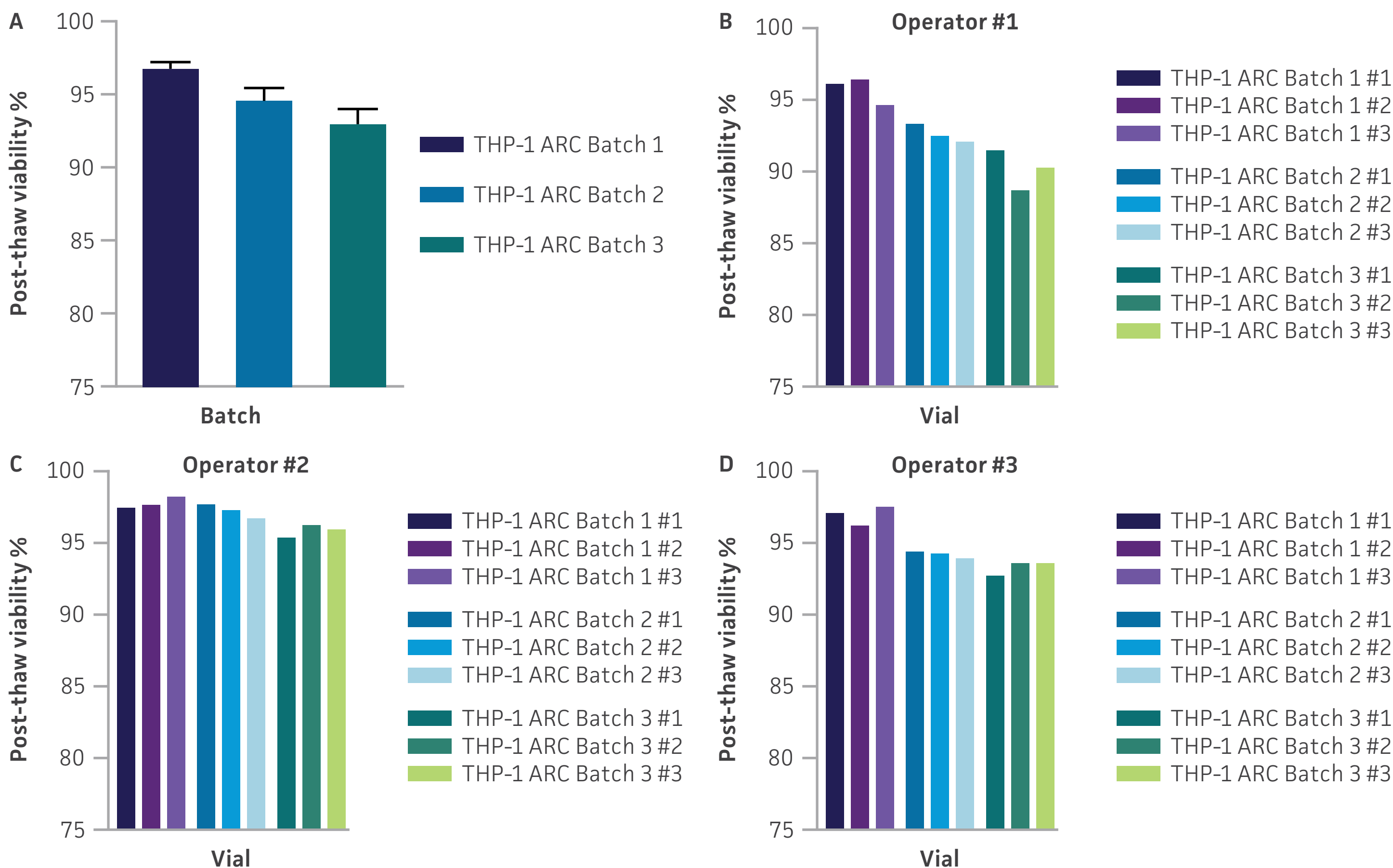



**Figure 2: Post-thaw viability of ThawReady™ THP-1 cells.** ThawReady™ THP-1 cells from three batches were thawed, and post-thaw viability was measured using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®). (A) Average post-thaw viability for three batches (combined data from 3 operators). (B) Post-thaw viability of individual vials measured by operator #1. (C) Post-thaw viability of individual vials measured by operator #2. (D) Post-thaw viability of individual vials measured by operator #3.

The human monocytic cell line THP-1 can be differentiated into macrophage-like cells using phorbol 12-myristate 13-acetate (PMA).[5] Differentiated macrophages have been widely used as in vitro models to study macrophage involvement in inflammatory responses.[5] To ensure the ThawReady™ THP-1 cells maintain the characteristics and functionality of parental THP-1 cells, 100 ng/mL PMA was used to stimulate differentiation of ThawReady™ THP-1 cells. Like the parental THP-1 cells, ThawReady™ THP-1 cells become adherent within 24 hours of PMA treatment and then continue to acquire a larger cytoplasmic volume and more granular appearance,[5] demonstrating their ability to differentiate into macrophage-like cells (Figure 3).

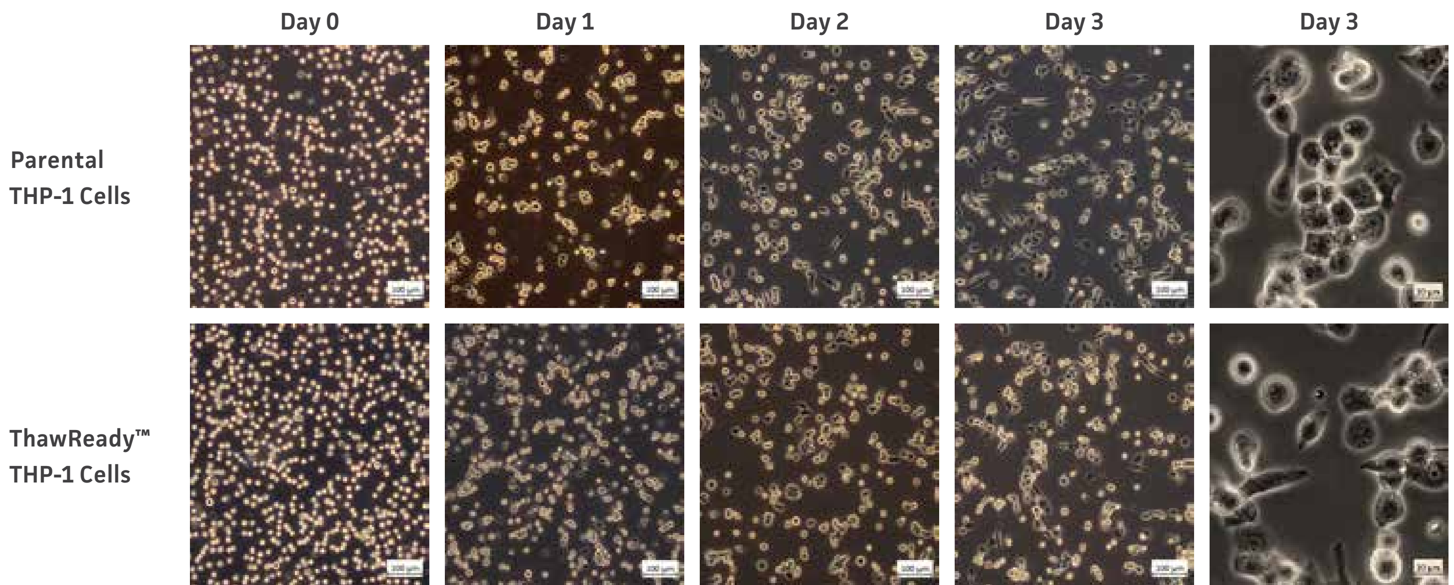



**Figure 3: Morphological changes of macrophage-like cells with differentiation.** Parental THP-1 and freshly thawed ThawReady™ THP-1 cells were plated and treated with PMA for 3 days to stimulate differentiation into macrophage-like cells. Cell morphology was observed under the microscope and cell images were captured using a digital camera on Day 0, Day 1, Day 2, and Day 3 after PMA stimulation (Day 3 high mag images highlight morphology of macrophage-like cells).

CD14, which is expressed on the surface of monocytes/macrophages, is often used as an indicator of THP-1 cell differentiation and its expression can increase with the induction with PMA.[6] CD36, which acts as a scavenger receptor, contributes to the uptake of oxidized low density lipoprotein (LDL) particles and fatty acids by macrophages, and participates in internalization of apoptotic cells.[3] CD36 is known to be regulated during THP-1 monocyte differentiation. To evaluate key marker gene expression differences between parental THP-1 and ThawReady™ THP-1 cells and their differentiated macrophages, CD14 and CD36 qPCR was carried out. Both the undifferentiated parental THP-1 and ThawReady™ THP-1 cells showed low-level expression of CD14 and CD36. Upon PMA stimulation, the expression of CD14 and CD36 was dramatically increased in the differentiated macrophage-like cells derived from both the parental THP-1 and ThawReady™ THP-1 cells as compared to their undifferentiated counterparts (Figure 4). These results demonstrate that the ThawReady™ THP-1 cells maintain the key marker gene expression characteristics of the parental THP-1 cells.

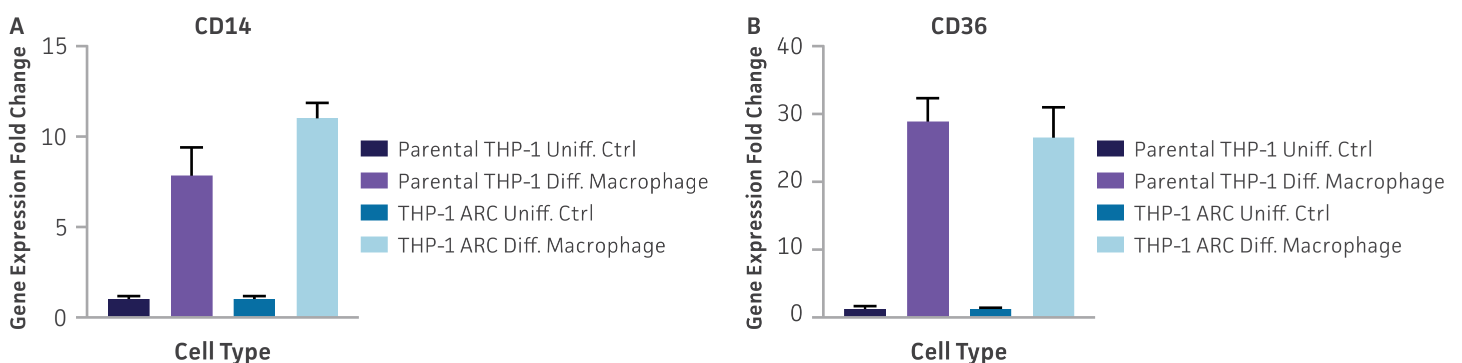



**Figure 4: Increased mRNA expression of CD14 and CD36 in PMA induced macrophage-like cells.** Parental THP-1 and freshly thawed ThawReady™ THP-1 cells were plated and treated with PMA for 3 days to stimulate differentiation into macrophage-like cells. qPCR was performed to quantify (A) CD14 and (B) CD36 mRNA expression. Upon PMA induction, mRNA expression of CD14 and CD36 in macrophage-like cells derived from both parental THP-1 cells and ThawReady™ THP-1 cells was significantly increased compared to the undifferentiated controls.

Initially characterized as a cell membrane protein, CD14 exists in both soluble (sCD14) and membrane-associated (mCD14) protein forms.[6] Flow cytometry analysis was carried out to evaluate the cell surface CD14 expression in both parental THP-1 and ThawReady™ THP-1 cells. CD14 was expressed on almost the entire cell populations of the parental THP-1 and ThawReady™ THP-1 cells as well as their differentiated macrophage-like cells (Figure 5).

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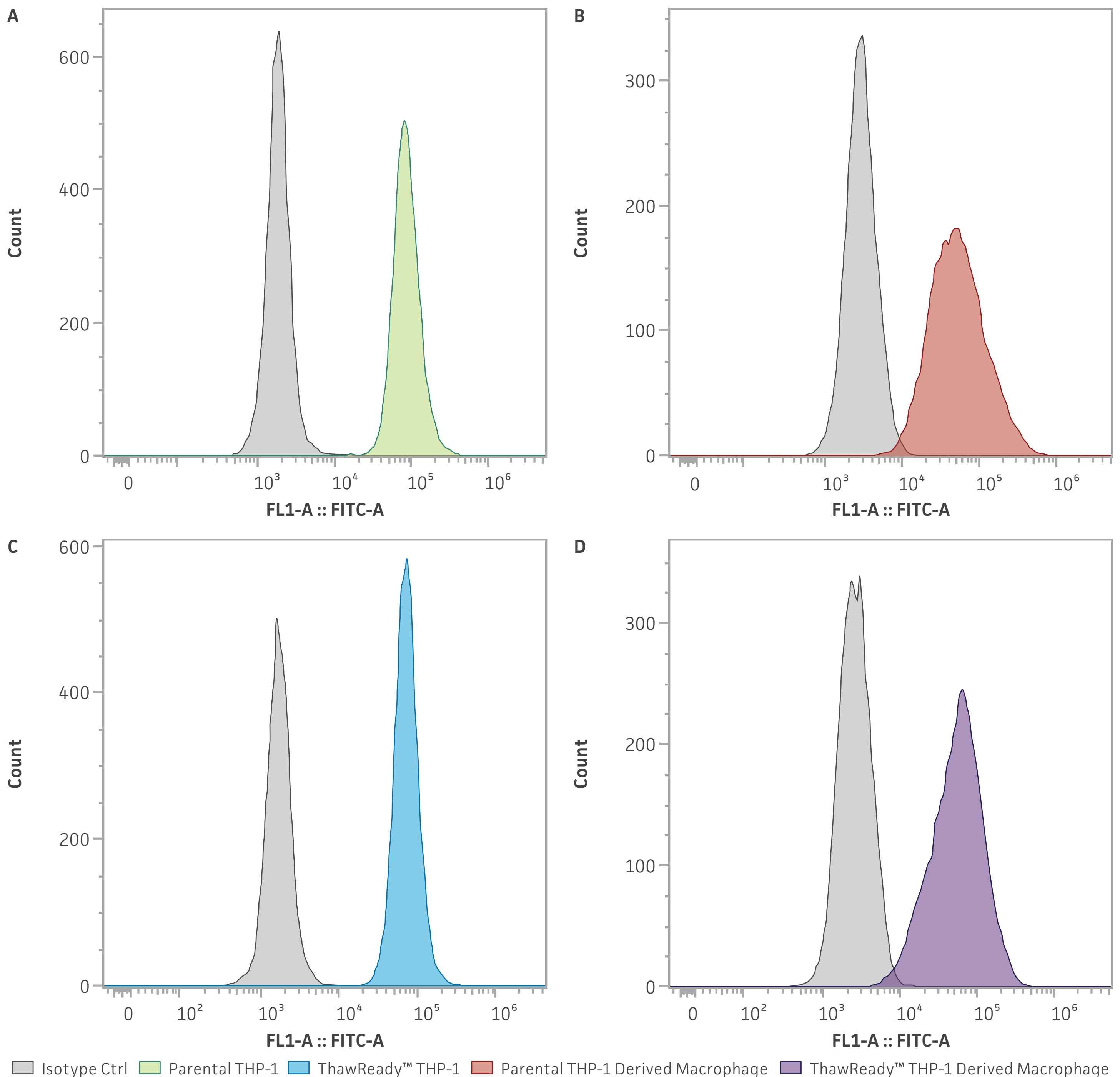


**Figure 5: CD14 cell surface protein expression analysis by flow cytometry.** Parental THP-1 and freshly thawed ThawReady™ THP-1 cells were plated and treated with PMA for 3 days to differentiate into macrophage-like cells. Cell surface expression of CD14 on (A) undifferentiated parental THP-1 cells, (B) differentiated macrophage-like cells derived from parental THP-1 cells, (C) undifferentiated ThawReady™ THP-1 cells, and (D) differentiated macrophage-like cells derived from ThawReady™ THP-1 cells were analyzed by flow cytometry (CytoFLEX®, Beckman Coulter®) using BD Pharmingen™ FITC Mouse Anti-Human CD14 antibody and BD Pharmingen™ FITC Mouse IgG2b κ Isotype Control (BD Biosciences).

Phagocytosis of pathogens, apoptotic cells, and debris are defining features of macrophage function.[4] The phagocytic capability of ThawReady™ THP-1–derived macrophage-like cells was assessed using pHrodo™ pathogen bioparticles (Thermo Fisher Scientific® #P35361), which only fluoresce when internalized in the acidic environment of the phagolysosome (Figure 6B). We used LysoTracker™ (Thermo Fisher Scientific® # L7526) to stain lysosomes, which accumulate in the cytoplasm with macrophage differentiation (Figure 6C). We found that the ingested pHrodo™ bioparticles in cells undergoing phagocytosis were co-localized with cellular lysosomes stained by LysoTracker™ (Figure 6D), indicating phagolysosome formation during phagocytosis. These data confirm that ThawReady™ THP-1 cells can effectively differentiate into macrophage-like cells that maintain phagocytic capacity.

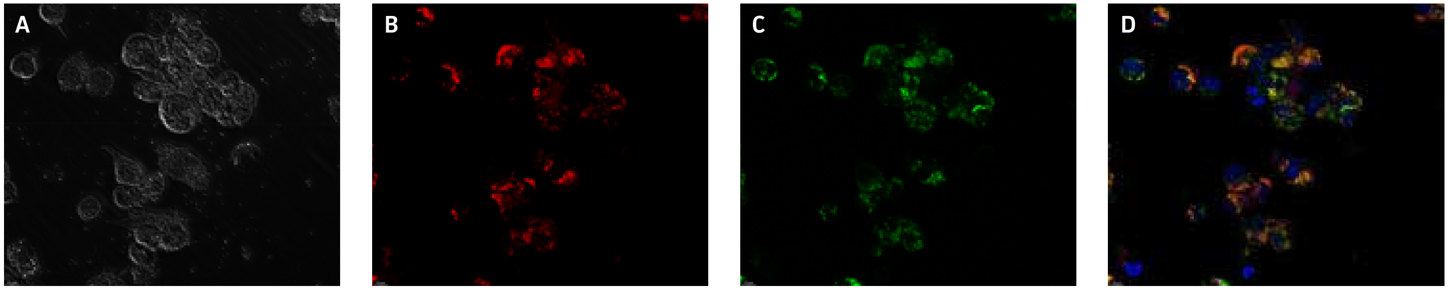


**Figure 6: Phagocytosis assay of ThawReady™ THP-1–derived macrophage-like cells.** Freshly thawed ThawReady™ THP-1 cells were incubated with PMA for 3 days to differentiate into macrophage-like cells. (A) Phase contrast image of the differentiated macrophage-like cells. (B) Cells undergoing phagocytosis with ingested pHrodo™ (red; Thermo Fisher Scientific®) bioparticles. (C) Cellular lysosomes stained with LysoTracker™ (green; Thermo Fisher Scientific®). (D) Overlay of pHrodo™ phagocytosis and LysoTracker™ lysosome staining images. DAPI (Thermo Fisher Scientific®) stained nuclei showed in blue.

## CONCLUSIONS

Leveraging our proprietary ABP-free cryopreservation media and well-established high-standard cell culture practices, ATCC has developed the highly functional ThawReady™ THP-1 cell product. It consistently exhibits high post-thaw viability with low intra-lot and inter-lot variation and shows the expected characteristics and functionality equivalent to the parental THP-1 cells. Our ThawReady™ THP-1 cells demonstrate consistency and reproducibility in achieving optimal performance in cell-based assays, offering advantages like long-term access to a consistent resource, more flexible scheduling, and cost savings. This novel product allows for extensive biopharmaceutical studies while avoiding the lengthy and costly development typically required with establishing cell-based assays.

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