Development of the *ThawReady*™ THP-1 Product for Cell-Based Assays

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Overview

In this study, we showcase the development of a novel assay-ready cell (ARC) product using the THP-1 cell line: ThawReady™ THP-1 (ATCC® TIB-202-AR™). We 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. 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. Additionally, traditional cell-based assays have several disadvantages such as the labor required to maintain cell culture, the cost of consumables, the need for laboratory space, and the use of dedicated equipment. These disadvantages 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™).

Methods

To establish the cell expansion process for 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 following ATCC® cell culture protocols. A proprietary animal by-product (ABP)-free cryomedium was developed to freeze the ThawReady™ THP-1 cells. Cell viability was measured using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®).

I. Overview of a Traditional Cell-Based Assay and a ThawReady™ Assay

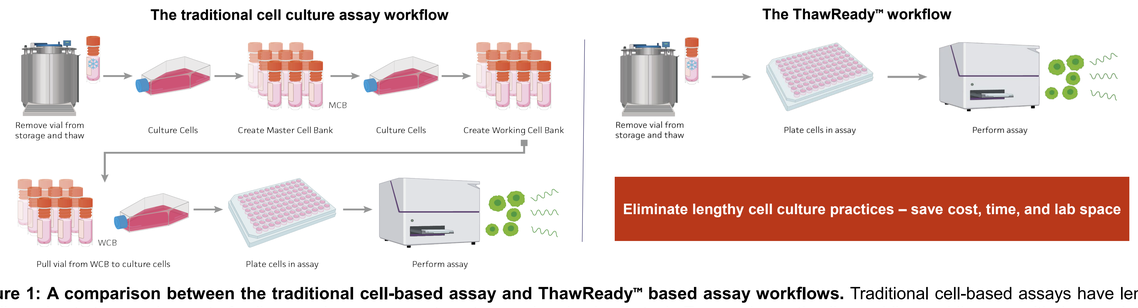

Figure 1: A comparison between the traditional cell-based assay and ThawReady™ based assay workflows. Traditional cell-based assays have lengthy timelines due to the requirement of cell expansion processes to get a synchronized cell stock. To speed your timelines while providing you with the consistency you need, ATCC developed a new product. ThawReady™ cells 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

II. Post-Thaw Viability of THP-1 ThawReady™ Cells

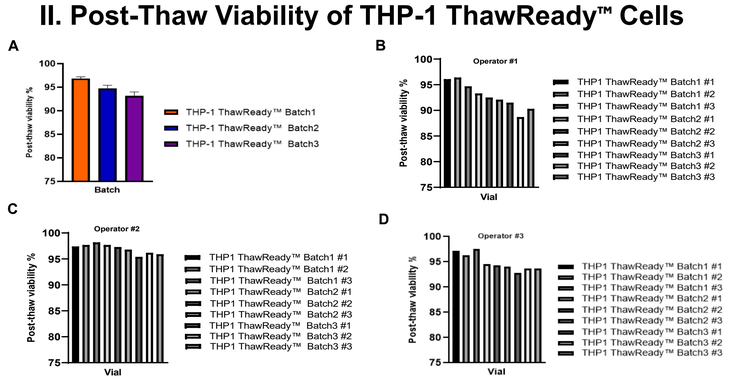

Figure 2: Post-thaw viability of THP-1 ThawReady™ cells. THP-1 ThawReady™ cells from three batches were thawed, and post-thaw viability was measured using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®). (A) Average post-thaw viability for three batches (combined data from 3 operators). (B)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

III. Morphological Changes of Macrophage-Like Cells with Differentiation

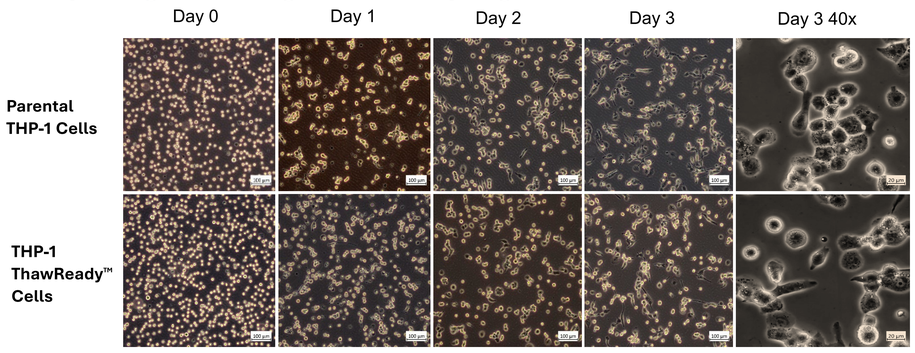

Figure 3: Morphological changes of macrophage-like cells with differentiation. Parental THP-1 and freshly thawed THP-1 ThawReady™ cells were plated and treated with Phorbol 12-myristate 13-acetate (PMA) for 3 days to induce differentiation into macrophage-like cells. Cell morphology was observed under 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 40x images highlight morphology of macrophage-like cells).

IV. Increased mRNA Expression of CD14 and CD36 in PMA-induced Macrophage-like Cells

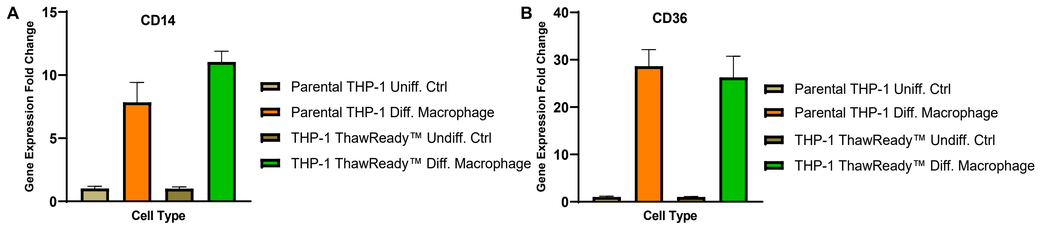

Figure 4: Increased mRNA expression of CD14 and CD36 in PMA induced macrophage-like cells. Parental THP-1 and freshly thawed THP-1 ThawReady™ cells were plated and treated with PMA for 3 days to differentiate into macrophage-like cells. qPCR was performed to quantify (A) CD14 and (B) CD36 mRNA expression.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

V. CD14 Cell Surface Protein Expression Analysis by Flow Cytometry

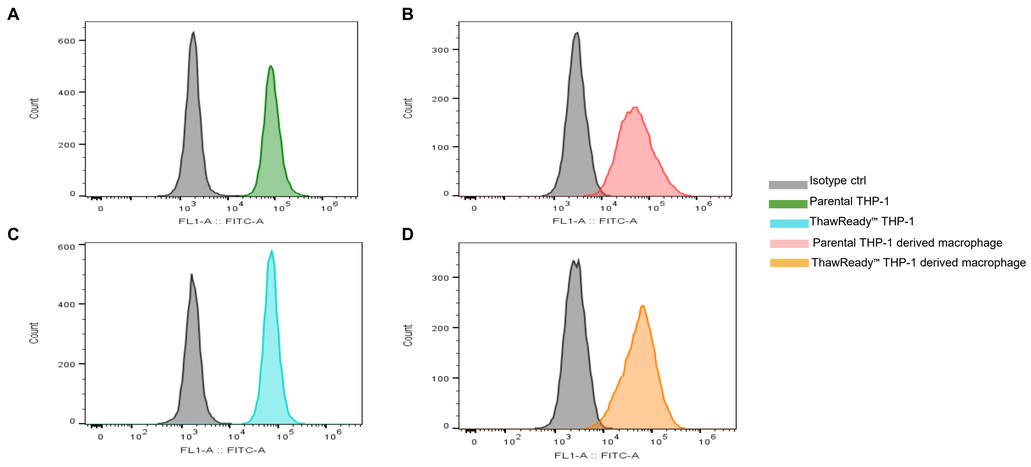

Figure 5: CD14 Cell Surface Protein Expression Analysis by Flow Cytometry. Parental THP-1 and freshly thawed THP-1 ThawReady™ cells were plated and treated with PMA for 3 days to differentiate into macrophage-like cells. Cell surface expression of CD14 on (A) undifferentiated parental THP-1 cells, (B)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

VI. Phagocytosis Assay of THP-1 ThawReady™-derived Macrophage-Like Cells

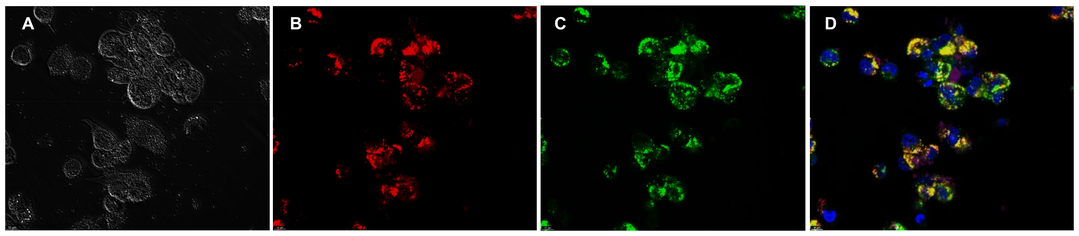

Figure 6: Phagocytosis assay of THP-1 ThawReady™-derived macrophage-like cells. Freshly thawed THP-1 ThawReady™ cells were incubated with PMA for 3 days to differentiate into macrophage-like cells. (A) Phase contrast image of the differentiated macrophage-like cells. (B) Cells undergoing phagocytosis with ingested pHrodo™ (red; Thermo Fisher Scientific®) bioparticles. (C) Cellular lysosomes stained with LysoTracker™ (green; Thermo Fisher Scientific®). (D). Ingested red pHrodo™ bioparticles in cells undergoing phagocytosis were co-localized with cellular lysosomes stained in green by LysoTracker™, indicating phagolysosome formation during phagocytosis. DAPI (Thermo Fisher Scientific®) stained nuclei showed in blue.

Conclusions

- Leveraging our proprietary animal by-product (ABP)-free cryopreservation media and well-established high-standard cell culture practices, ATCC® has developed a highly functional THP-1 ThawReady™ product (ATCC® TIB-202-AR™).
- 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 THP-1 ThawReady™ cells demonstrate consistency and reproducibility in achieving optimal performance in cell-based assays, offering advantages including long-term access to a consistent resource, more flexible scheduling, and cost savings, allowing for extensive biopharmaceutical studies while avoiding the lengthy and costly development typically required for establishing cell-based assays.

References:

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