Development of a THP-1 Assay Ready Product for Cell-Based Assays

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Introduction

Cell-based assays are extensively used in both research and industry settings for a wide variety of applications including target identification, drug development, and compound toxicity testing. A major challenge when using cell-based assays is the inherent variability of cultured cells. The parameters that contribute to this variability include cell culture practice, phenotypic drift associated with long-term cell cultivation, and biomaterials resourced from different labs. The disadvantages of maintaining continuous culture have driven the need for cell products that are ready for immediate use in cell-based assays.

Here, we report the development of Assay Ready THP-1 (ATCC® TIB-202-AR™), a standardized assay-ready cell (ARC) product utilizing the THP-1 (ATCC® TIB-202™) cell line—a commonly used and physiologically relevant cell model that is known to be difficult to handle. During product development and manufacturing, high standards and strict cell culture processes were used for maintaining the characteristics and authenticity of the culture, and a proprietary animal by-product (ABP)-free cryomedium was developed to freeze the cells.

ATCC® TIB-202-AR™ was manufactured to meet the defined specifications for low intra-lot and inter-lot variation and demonstrates repeatability in achieving optimal performance in cell-based assays. It consistently exhibits high viability, fast recovery post-thaw, and the ability to differentiate into macrophage-like cells that express the appropriate macrophage markers and display the expected functional attributes. Our THP-1 ARC product offers improvements in use of laboratory resources, experiment scheduling, and assay robustness and variability, allowing for extensive study of biopharmaceuticals while avoiding the lengthy and costly development normally required for establishing cell-based assays.

Results

I. Overview of a Traditional Cell-Based Assay and an ARC-based Assay

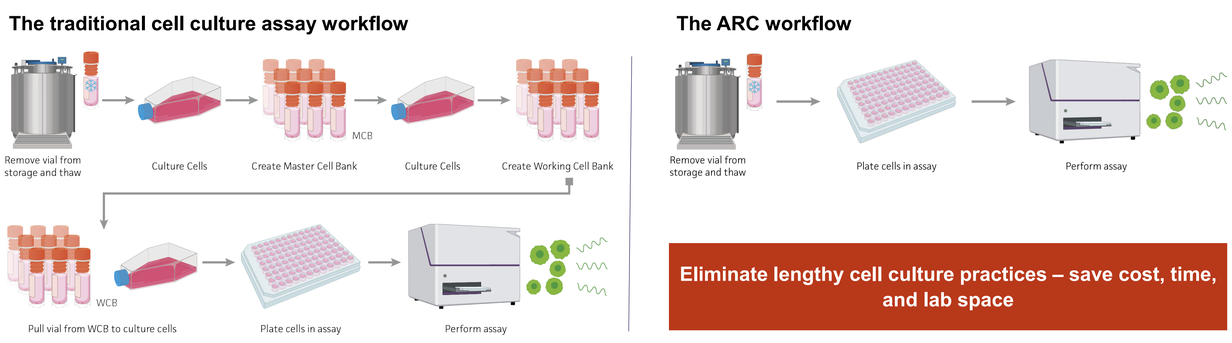

Figure 1: A comparison between the traditional cell-based assay and ARC-based assay workflows. Traditional cell-based assays have lengthy timelines due to the requirement of cell expansion processes to get a synchronized cell stock. To speed your timelines while providing you with the consistency you 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.

II. Post-Thaw Viability of THP-1 Assay Ready Cells

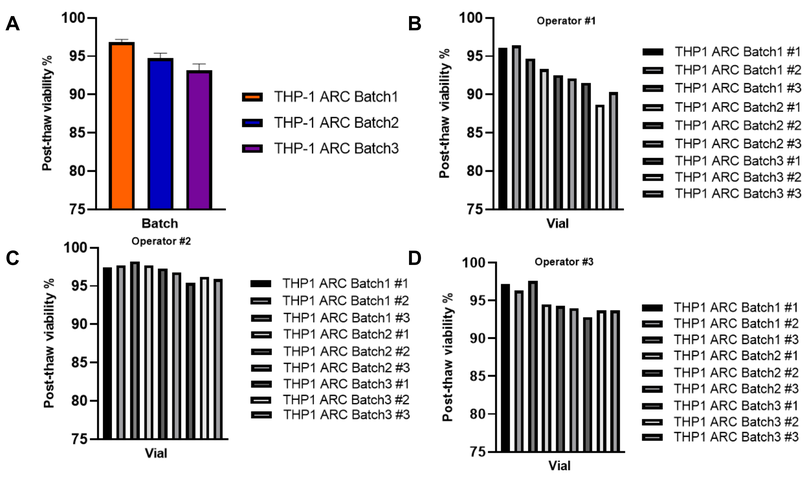

Figure 2: Post-thaw viability of THP-1 Assay Ready Cells. THP-1 Assay Ready Cells from three batches were thawed, and post-thaw viability was measured using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®). (A) Average post-thaw viability for three batches (combined data from 3 operators). (B)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

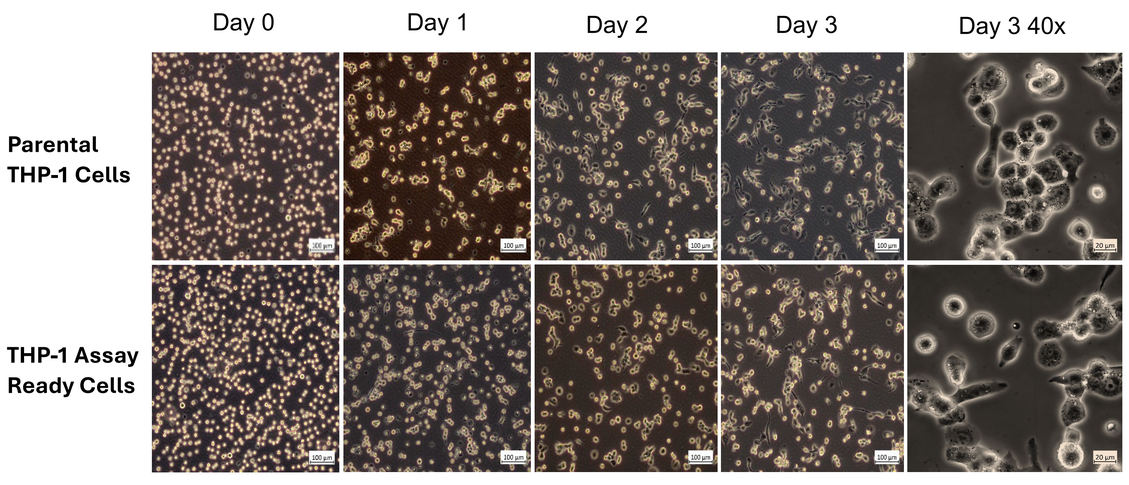

Figure 3: Morphological changes of macrophage-like cells with differentiation. Parental THP-1 and freshly thawed THP-1 Assay Ready cells were plated and treated with Phorbol 12-myristate 13-acetate (PMA) for 3 days for differentiation into macrophage-like cells. Cell morphology was observed under the 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

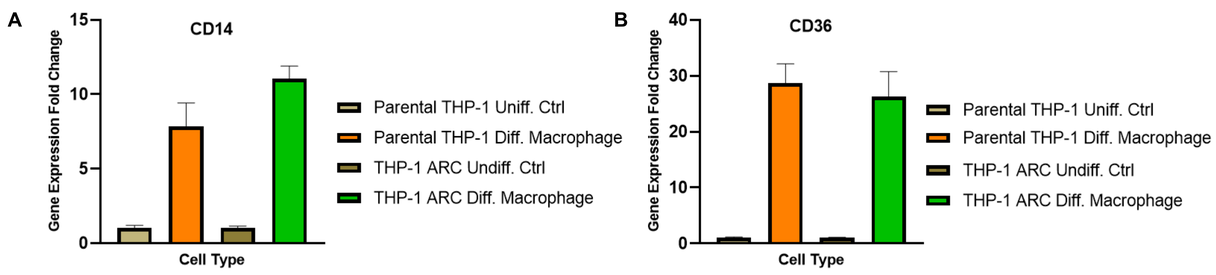

Figure 4: Increased mRNA expression of CD14 and CD36 in PMA induced macrophage-like cells. Parental THP-1 and freshly thawed THP-1 ARCs were plated and treated with PMA for 3 days to differentiate into macrophage-like cells. qPCR was performed to quantify (A) CD14 and (B) CD36 mRNA expression. Upon PMA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

V. CD14 Cell Surface Protein Expression Analysis by Flow Cytometry

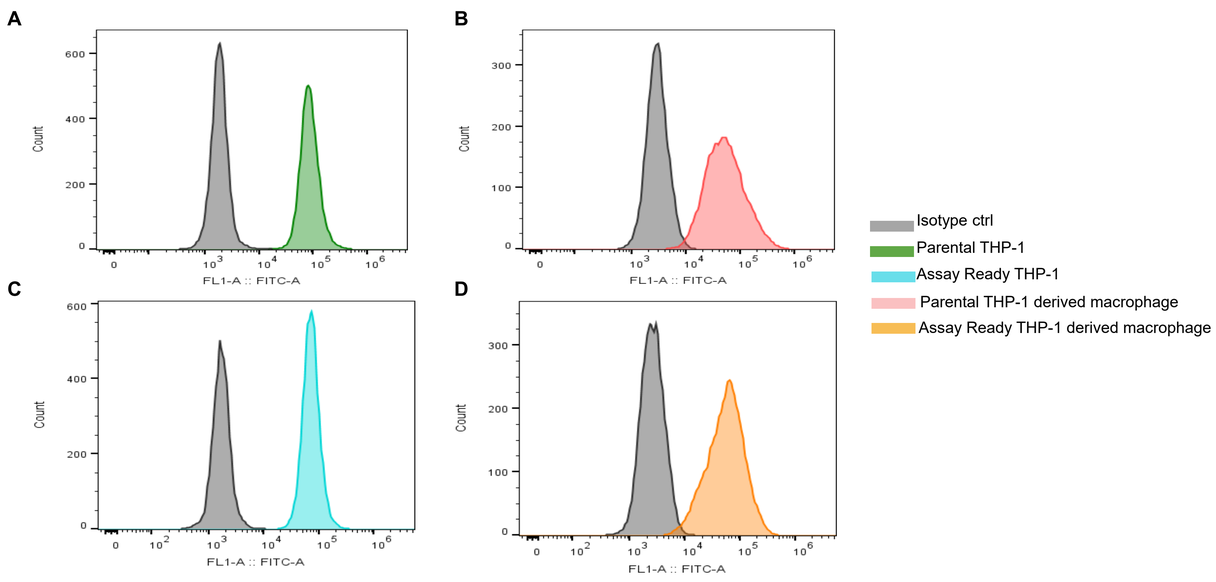

Figure 5: CD14 Cell Surface Protein Expression Analysis by Flow Cytometry. Parental THP-1 and freshly thawed THP-1 Assay Ready cells were plated and treated with PMA for 3 days to differentiate into macrophage-like cells. Cell surface expression of CD14 on (A) undifferentiated parental THP-1 cells, (B) differentiated macrophage-like cells derived from parental THP-1 cells, (C) undifferentiated THP-1 Assay Ready cells, and (D) differentiated macrophage-like cells derived from THP-1 Assay Ready 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 ARC-derived Macrophage-Like Cells

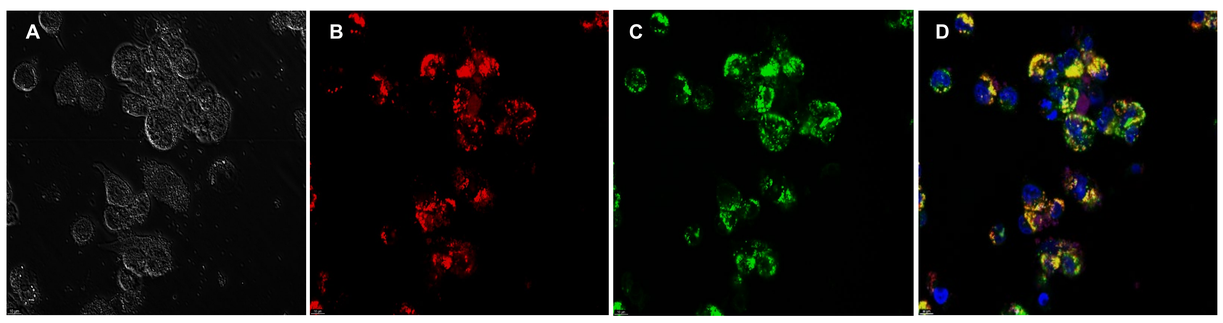

Figure 6: Phagocytosis assay of THP-1 ARC-derived macrophage-like cells. Freshly thawed THP-1 Assay Ready cells were incubated with PMA for 3 days to differentiate into macrophage-like cells. (A) Phase contrast image of the differentiated macrophage-like cells. (B) Cells undergoing phagocytosis with 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 ATCC® TIB-202-AR™, a highly functional THP-1 ARC 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 THP-1 ARCs 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 with establishing cell-based assays.

References:

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