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APPLICATION NOTE

*Thaw***Ready**™ by ATCC

Accelerate Cell-Based Assays with the ThawReady™ THP-1 NF-κB-Luc2 Reporter Line

Rajnee Kanwal, PhD; Diana Douglas, BS; Joshua Franklin, BS; Lukas Underwood, PhD; Jacqueline Mikhaylov, BS; Quinn Osgood, MS; Nilay Chakraborty, PhD; Fang Tian, PhD; Weiguo Shu, PhD
ATCC, Manassas, VA 20110

ABSTRACT

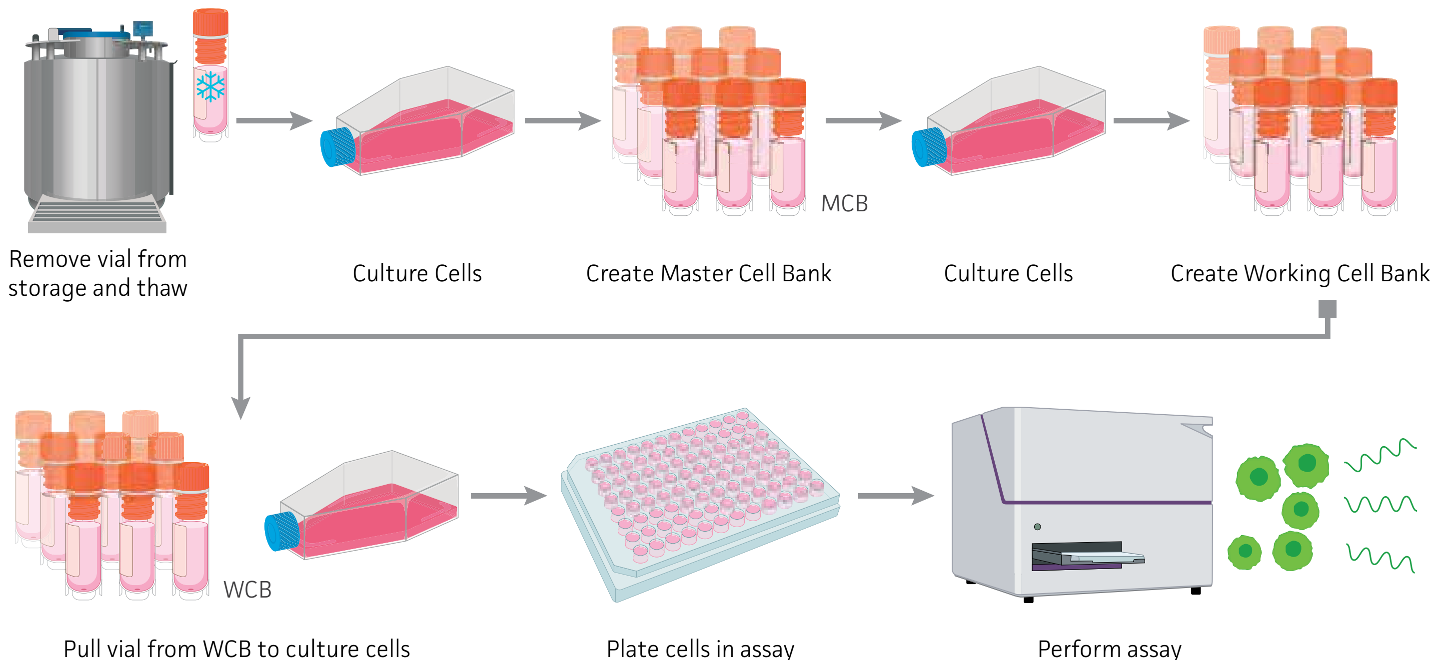
Cell-based assays are pivotal in the drug development process and are widely used in drug discovery screening to measure cytotoxicity, biological activity, biochemical mechanisms, and off-target effects. However, the inherent variability of cultured cells poses a significant challenge. To address this, ATCC has developed an assay-ready cell (ARC) product using the THP-1 NF-κB-Luc2 cell line: ThawReady™ THP-1 NF-κB-Luc2 (ATCC® TIB-202-NFkB-LUC2-AR™). The THP-1 NF-κB-Luc2 cell line was originally developed by modifying THP-1 monocytes to stably express the firefly luciferase gene under the control of an NF-κB responsive element. This model has been extremely useful for cell-based assays as evaluation of luciferase expression changes can serve as a sensitive indicator of NF-κB regulation levels during drug screening studies.[1] By further optimizing this cell line into an ARC product, we are providing an innovative solution that saves time and resources, improves data quality, reduces batch-to-batch variations, and increases flexibility in usage. In this study, we will showcase the development of this novel product and demonstrate that it consistently exhibits high post-thaw viability and maintains the desired functionality.

INTRODUCTION

Cell-based assays are used in basic life science research and various stages of drug development such as target identification and compound toxicity testing. These assays generally exhibit higher variability than biochemical-based methods mainly due to inherent variations in cell culture resulting from contamination or phenotypic drift associated with long-term cultivation.[2,3] The cultivation of cells itself also involves labor-intensive lab procedures that can impact the quality and functionality of the cells. Standardization of the assay protocols, reagents, and biological materials used in cell-based assays is therefore essential for achieving reliable assay performance.

The limitations of maintaining continuous culture have led to a demand for readily available cells for immediate use in cell-based assays. Assay-ready cells (ARCs) meet this demand, offering significant advantages like time and resource savings, improved data quality, reduced batch-to-batch variation, and increased flexibility (Figure 1).

THE TRADITIONAL CELL CULTURE WORKFLOW



THE THAWREADY™ WORKFLOW

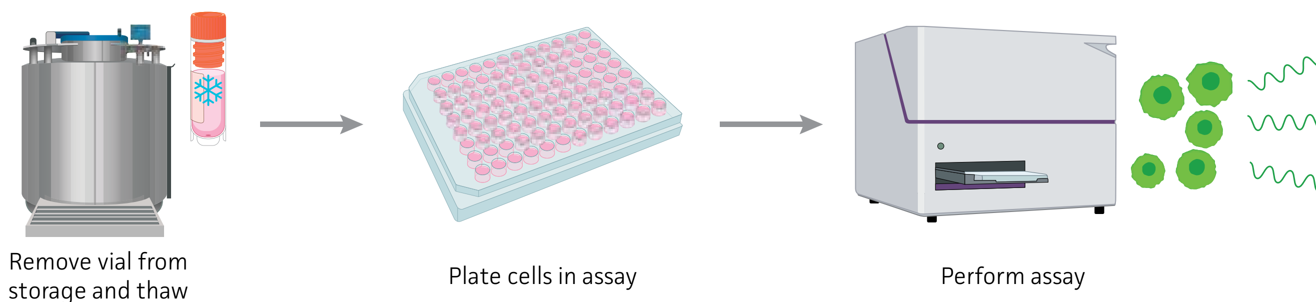


Figure 1: Overview of the traditional cell-based assay and ARC-based assay workflows. Traditional cell-based assay workflows require lengthy cell expansion processes for preparing cells. ARCs are ready within hours of thawing and are scalable for high-throughput assays, thereby streamlining workflows by months.

Leveraging our proprietary animal by-product (ABP)-free cryopreservation medium and well-established cell culture practices, ATCC has developed a THP-1 NF-κB-Luc2 ARC product: ThawReady™ THP-1 NF-κB-Luc2 (ATCC® TIB-202-NFkB-LUC2-AR™). Here, we optimized the culture and freezing conditions to provide cells with high post-thaw viability and functionality. These ARCs can be used directly from liquid nitrogen storage by simply thawing and seeding into assay plates, thereby eliminating lengthy cell expansion processes and accelerating pharmaceutically active compound discovery, biocompatibility testing, assay development, and cell signaling and disease pathway studies.

RESULTS WITH MATERIALS AND METHODS

POST-THAW VIABILITY EVALUATION

To develop ThawReady™ THP-1 NF-κB-Luc2 cells, THP-1 NF-κB-Luc2 cells (ATCC® TIB-202-NFkB-LUC2™) were cultivated in RPMI-1640 medium (ATCC® 30-2001™) supplemented with 10% Fetal Bovine Serum (ATCC® 30-2020™), 0.05 mM of 2-Mercaptoethanol (Thermo Fisher Scientific® #21985-023), and 1.0 µg/mL Puromycin (Thermo Fisher Scientific® #A1113803) following ATCC's thawing and culture processes. 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.

After cultivation, cells were harvested and cryopreserved with ATCC's proprietary ABP-free cryopreservation medium using a CryoMed® Controlled-Rate Freezer (Thermo Fisher Scientific®). The frozen cells were transferred to the vapor phase of liquid nitrogen (LN2) for long-term storage. Three batches of ThawReady™ THP-1 NF-kB-Luc2 cells were thawed, and post-thaw viability was measured using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®). All batches consistently exhibited high post-thaw viability with minimal variation within and between lots (Figure 2).

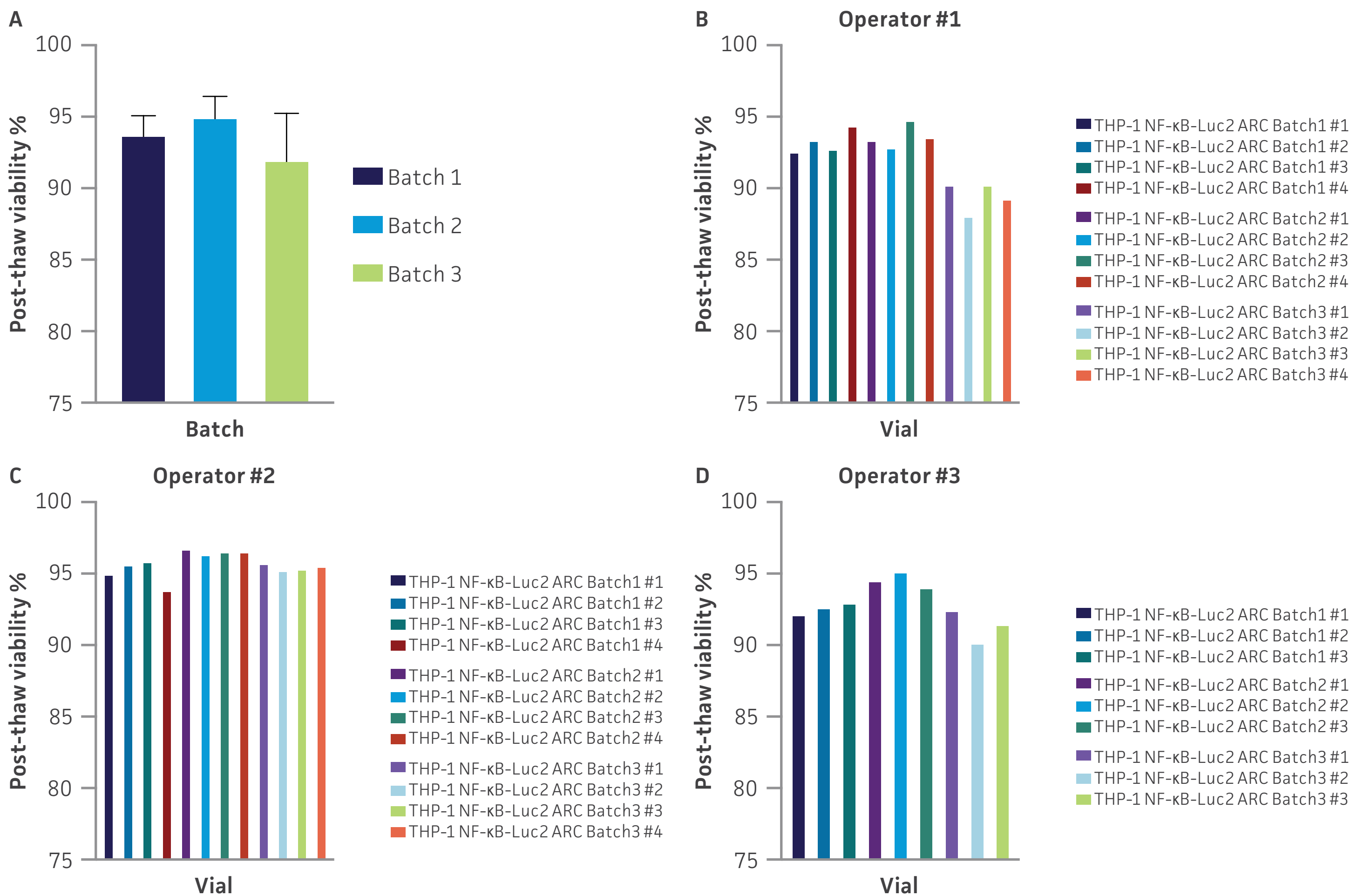


Figure 2: Post-thaw viability of ThawReady™ THP-1 NF-κB-Luc2 cells. ThawReady™ THP-1 NF-κB-Luc2 cells from three distinct batches were thawed, and post-thaw viability was determined using a Vi-CELL® BLU cell viability analyzer (Beckman Coulter®). (A) Average post-thaw viability for three batches (combined 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.

FUNCTIONAL EVALUATION OF THAWREADY™ THP-1 NF-κB-LUC2 CELLS

Lipopolysaccharide (LPS) is a ligand known to potently induce a proinflammatory immune response via activation of the NF-κB signaling pathway.[4] To evaluate NF-κB reporter activity, ThawReady™ THP-1 NF-κB-Luc2 cells were thawed, seeded, and stimulated with LPS at 50 ng/mL for 3 hours. After stimulation, cells were diluted to 1.0×10^5 cells/mL and transferred into 96-well opaque plates. Bright-Glo™ Luciferase Assay Reagent (Promega®) was added, and luminescence was measured using the Promega® GloMax® Explorer. Cells were assessed based on the average fold increase of luminescence (RLU) of LPS-stimulated cells relative to the average RLUs from the untreated controls. Upon LPS stimulation, the luciferase activity in stimulated cells increased at least 200-fold (Figure 3).

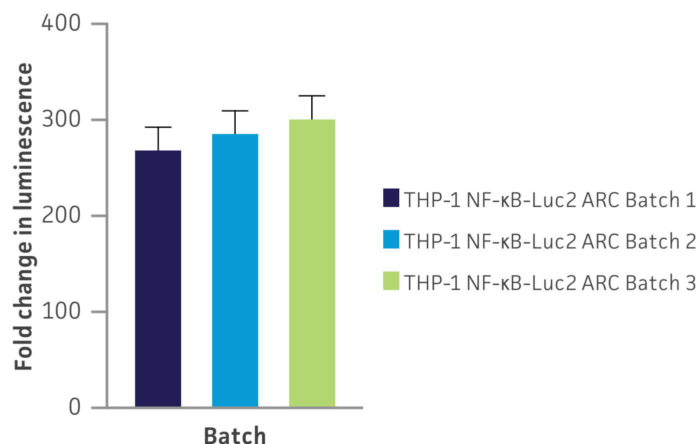


Figure 3: Assessment of luciferase reporter activity in ThawReady™ THP-1 NF-κB-Luc2 cells after LPS stimulation. ThawReady™ THP-1 NF-κB-Luc2 cells from three batches were thawed and seeded in 96-well plates then incubated with LPS at 50 ng/mL for 3 hours. The bioluminescence signal of the cells was detected using Bright-Glo™ (Promega®) and a Glomax™ luminometer (Promega®). Expression of luciferase in LPS-treated cells is displayed as fold change in bioluminescence signal intensity relative to LPS untreated controls.

ASSESSING THE EFFECTS OF POST-THAW REST TIME ON REPORTER ACTIVITY IN THAWREADY™ THP-1 NF-κB-LUC2 CELLS

To evaluate if extended post-thaw rest time affects NF-κB reporter activity, ThawReady™ THP-1 NF-κB-Luc2 cells were thawed, seeded, and allowed to rest for eight hourly time points. The cells were then stimulated with LPS at 50 ng/mL for 3 hours, and NF-κB reporter activity was measured (Figure 4). The study demonstrated that the NF-κB reporter retains sensitivity even at eight hours post-thaw rest. These cells showed an over 200-fold increase in signaling upon LPS stimulation, supporting that the ThawReady™ THP-1 NF-κB-Luc2 cells are ready to use for assaying upon thawing and are suitable for a one-day workflow.

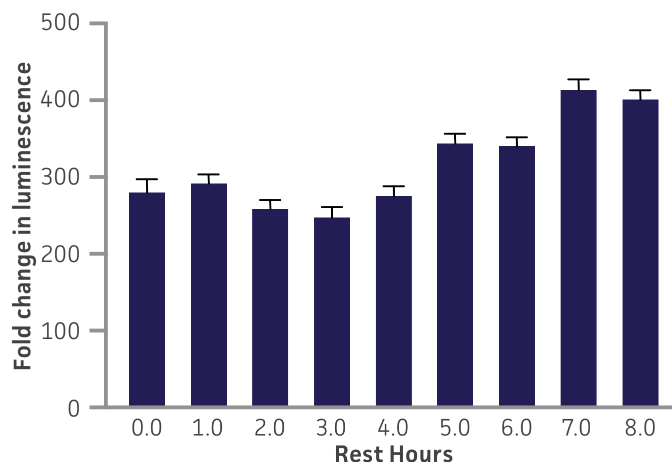


Figure 4: Evaluating the impact of post-thaw time on reporter activity in ThawReady™ THP-1 NF-kB-Luc2 cells. ThawReady™ THP-1 NF-κB-Luc2 cells were thawed and immediately seeded into 96-well plates or allowed to rest for the indicated time points. The cells were then incubated with LPS at 50 ng/mL for 3 hours. Bioluminescence signal of the cells was detected using Bright-Glo™ (Promega®) and a Glomax™ luminometer (Promega®). Expression of luciferase in LPS-treated cells is displayed as fold change in bioluminescence signal intensity relative to LPS untreated controls.

DOSE-DEPENDENT RESPONSE TO COMPOUND TREATMENT IN THAWREADY™ THP-1 NF-κB-LUC2 CELLS

Both parental THP-1 NF-κB-Luc2 cells and ThawReady™ THP-1 NF-κB-Luc2 cells were exposed to serial dilutions of LPS, and luminescence was measured. Increased NF-κB activity was observed in response to LPS in a dose-dependent manner in a similar pattern for both types of cells (Figure 5A). Additionally, cells were treated with the NF-κB inhibitor Bay 11-7182, which decreased luciferase activity in a dose-dependent manner in a similar pattern for both cell types (Figure 5B).

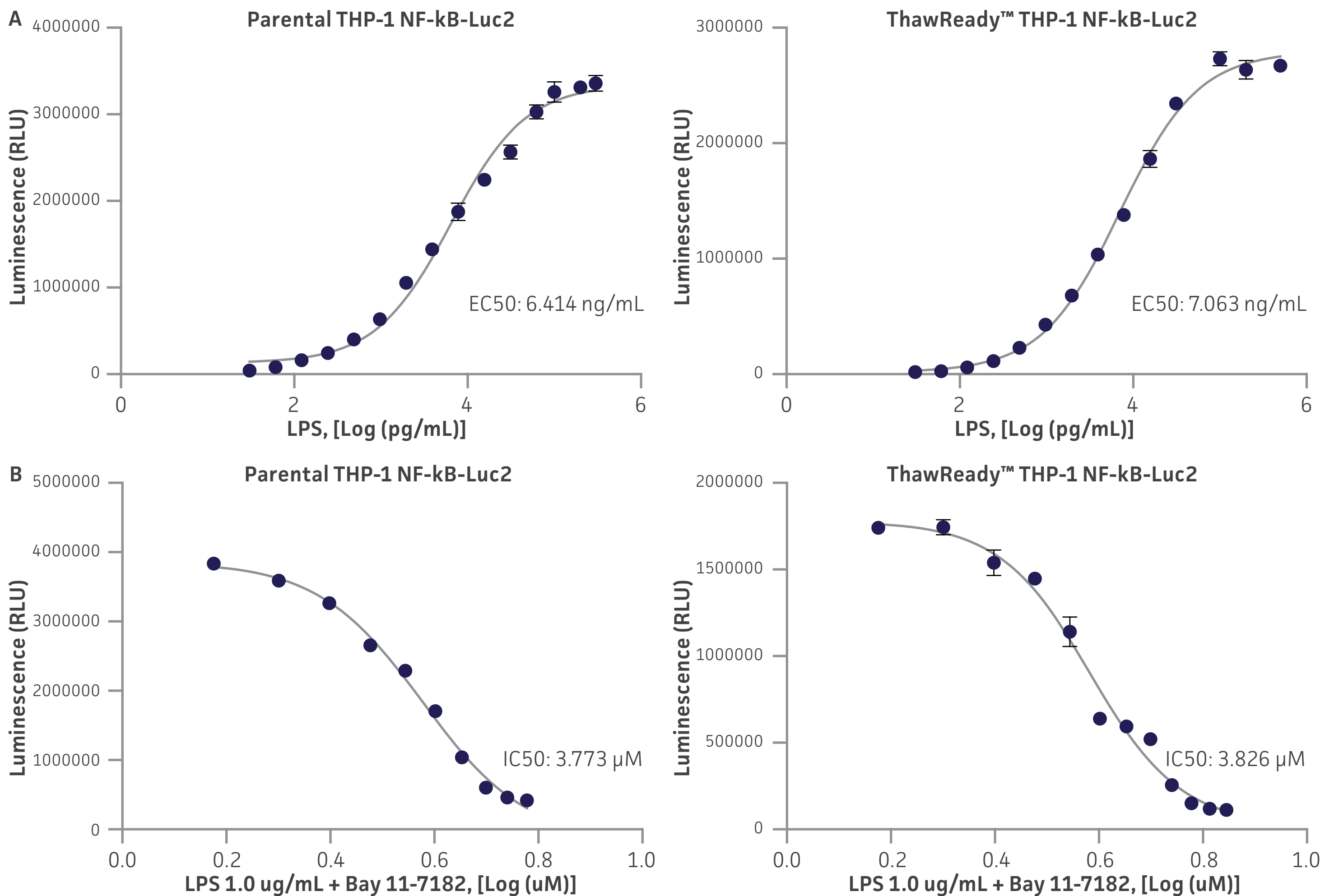


Figure 5: Dose-dependent response of bioluminescence signal to LPS and NF-κB inhibitor treatment. THP-1 NF-κB-Luc2 parental cells and post-thaw ThawReady™ THP-1 NF-κB-Luc2 cells were seeded into 96-well plates. After 2 hours of recovery, cells were treated with (A) a series of concentrations of LPS (MilliporeSigma®, L2880) or (B) 1.0 μg/mL of LPS and a series of concentrations of BAY 11-7182. After 3 hours treatment, bioluminescence signal of the cells was detected using Bright-Glo™ (Promega®) and a Glomax® luminometer (Promega®).

MORPHOLOGICAL ASSESSMENT OF THAWREADY™ THP-1 NF-κB-LUC2 CELLS

To assess whether the ThawReady™ THP-1 NF-κB-Luc2 cells maintain the characteristics of parental THP-1 NF-κB-Luc2 cells, the ARCs were thawed, seeded, and treated with PMA to induce differentiation into macrophage-like cells. PMA treatment led to cell adhesion, stellate morphology, and increased granularity and cytoplasmic volume.[5,6] (Figure 6).

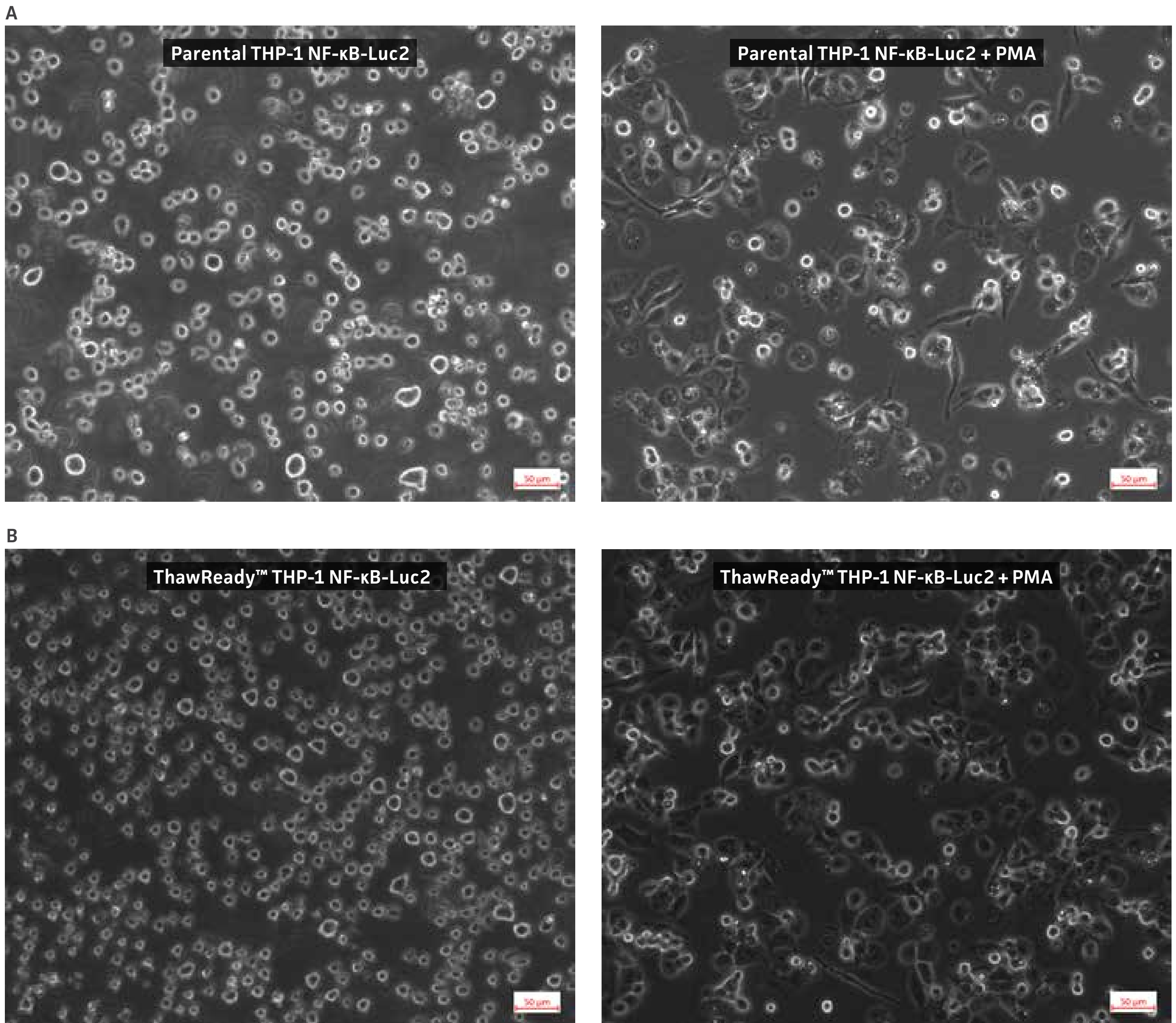


Figure 6: Morphological changes of ThawReady™ THP-1 NF-κB-Luc2 cells after PMA treatment. (A) Parental THP-1 NF-κB-Luc2 cells and (B) freshly thawed ThawReady™ THP-1 NF-κB-Luc2 cells were seeded and treated with 100 ng/mL PMA for 3 days. Morphological changes of the cells during the PMA treatment were captured using a digital camera. Images captured at day 3 after PMA treatment showed that both parental THP-1 NF-κB-Luc2 cells and ThawReady™ THP-1 NF-κB-Luc2 cells exhibited a macrophage-like morphology.

CONCLUSION

Cell culture is a time-consuming process that can add variability to cell-based assays. Using proprietary cryopreservation media and highly standardized cell culture practices, ATCC has developed an easy-to-use, highly functional cell line to accelerate cell-based assays: ThawReady™ THP-1 NF-κB-Luc2 (ATCC® TIB-202-NFkB-LUC2-AR™). These cells can be used directly as a standardized "reagent" in cell-based assays, eliminating the need for additional culturing. Our cells consistently exhibit high post-thaw viability with minimal intra-lot and inter-lot variation and offer functionality comparable to the parental THP-1 NF-κB-Luc2 cells. Incorporating our highly sensitive and reliable ThawReady™ THP-1 NF-kB-Luc2 cells into experiments will simplify your research processes and offer exceptional consistency, convenience, and efficiency.

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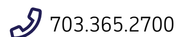

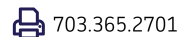

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