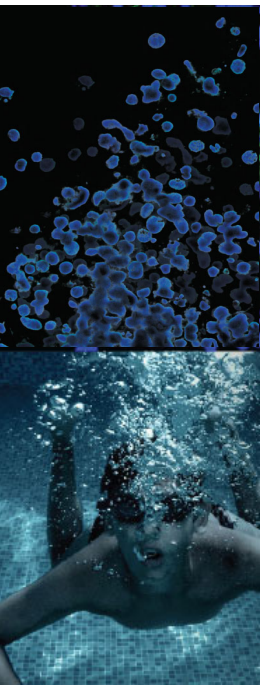
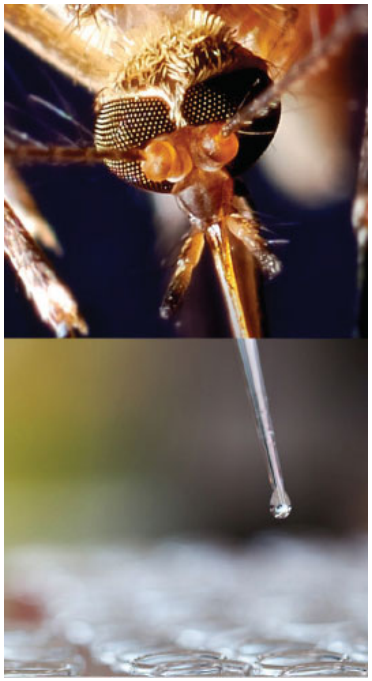
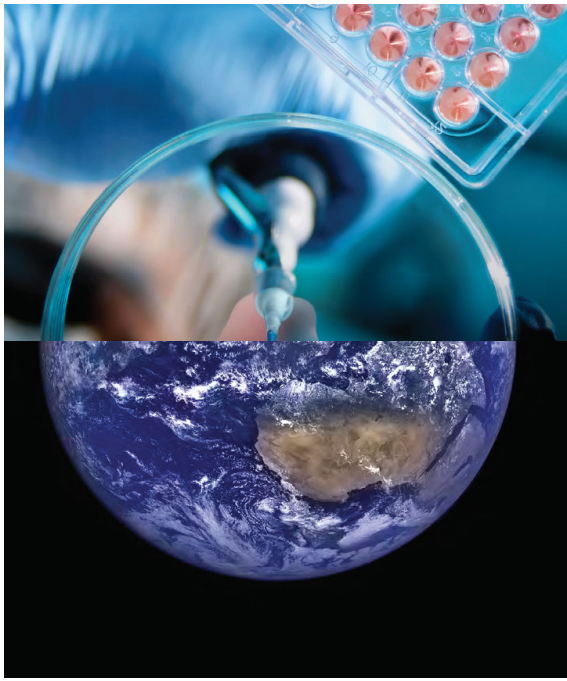
Advancements in Human Cell Line Cryopreservation for Assay Ready Efficiency

Lukas Underwood, Ph.D.
Diana Douglas, B.S.







Credible Leads to Incredible™

About ATCC®

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents
- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 500+ employees, over one-third with advanced degrees

ATCC®

Biological formats enabled by advanced bio-preservation

A new ATCC initiative focused on user convenience

- Led by Dr. Nilay Chakraborty
- Team of 13 biologists, scientists, and engineers with diverse scientific backgrounds and expertise
- Fast paced, strategic technology and product development

MicroQuant™ by ATCC®

Assay Ready Cells
From frozen to data in 1 day

ATCC®

Presentation overview

- Background information
  - Importance of in vitro bioassays and immortalized cell lines
  - Cell culture
- Industry pain point for performing bioassays
- Defining the complex commercial solution
- ATCCs Assay Ready solution and differentiating features
- Introduction of our first to Assay Ready conversions
- Presentation of application data
- Conclusion and call to action

In vitro bioassays and immortalized cell lines

FDA Modernization Act 2.0 allows for alternatives to animal testing

Cell-based bioassay uses

- High throughput screening of large chemical libraries
- Mechanism of action studies
- Cytotoxicity and safety screening
- Potency assays for quality control and product release testing

Benefits of immortalized cell lines

- Ease of use
- Global availability and extensive use
- Cost effectiveness
- Reproducibility and standardization

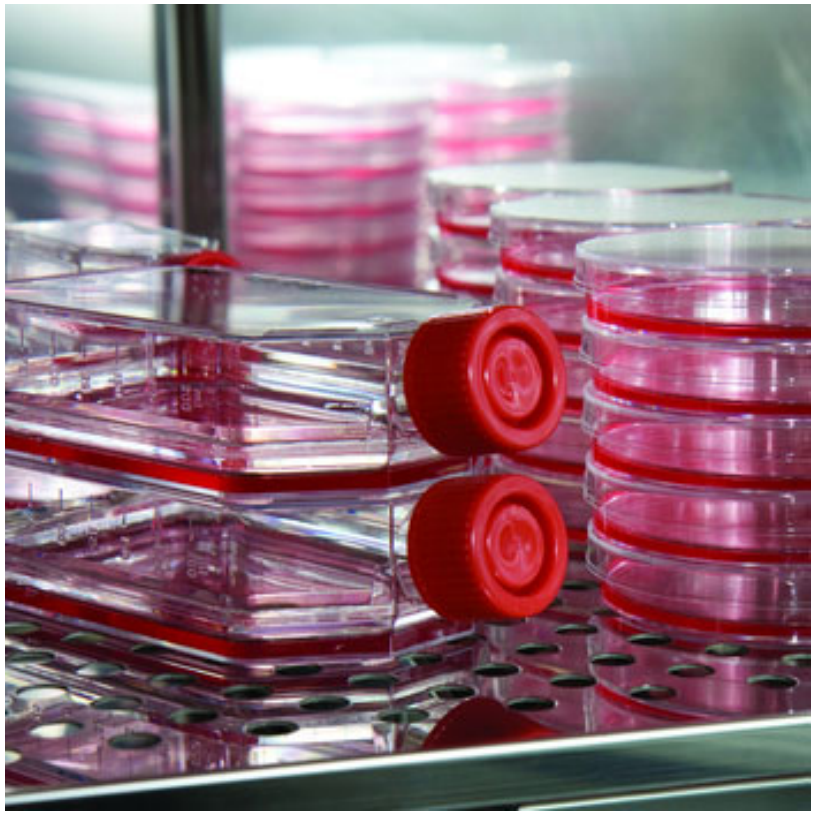
The insights gained from bioassays featuring immortalized cell lines build a foundation for subsequent testing in more expensive, less available advanced in vitro and animal models

ATCC

Cell culture

An industry staple and pain point?

- Fundamental in vitro research technique
- Provides a controlled environment that mimics natural in vivo conditions
- Facilitates maintenance, growth, and assessment of cells



Drawbacks

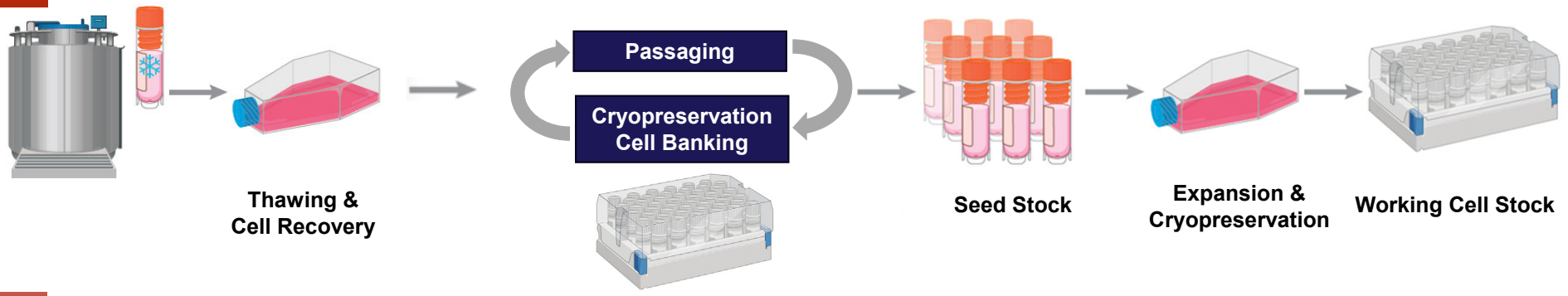
- Time consuming
- Cost and resource dependence
- Promotes variability

ATCC

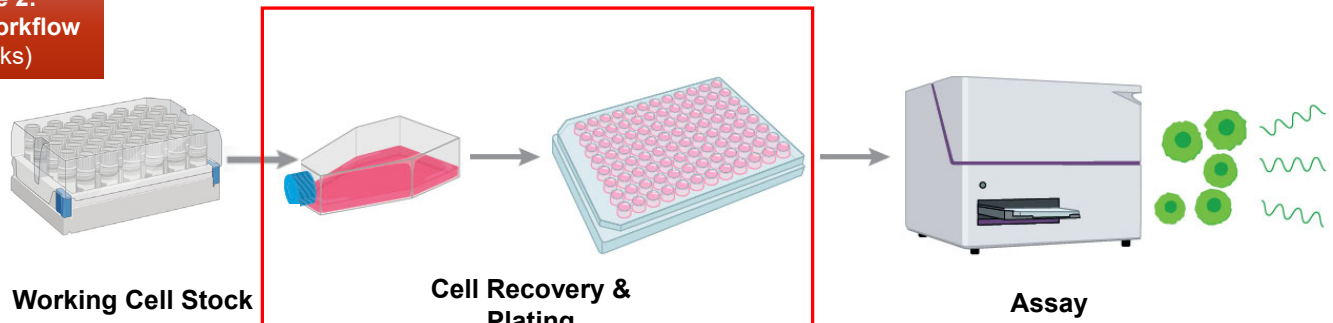
Problem 1: Cell culture for bioassays

Current practice

Phase 1:
Cell Banking
(Months)



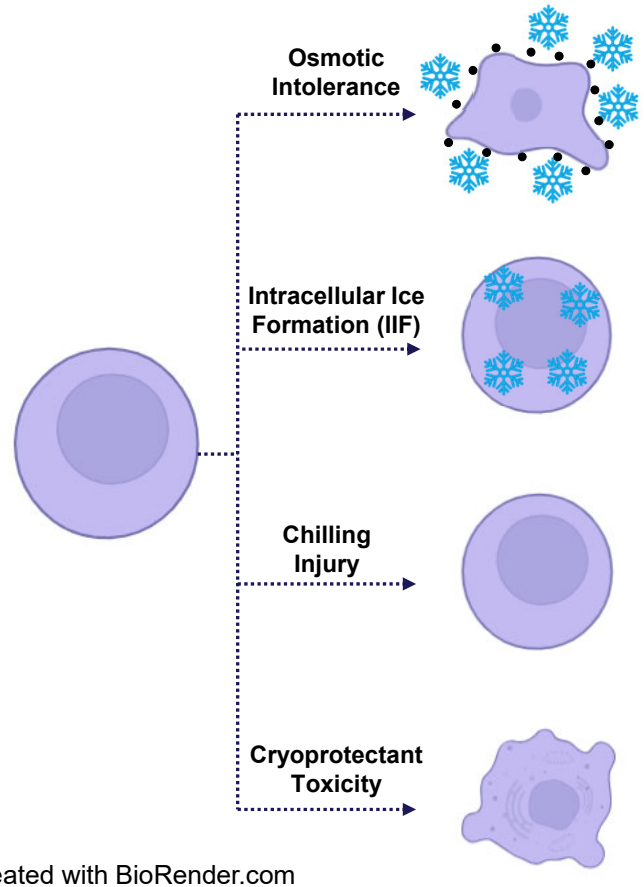
Phase 2:
Assay Workflow
(Weeks)



Created with BioRender.com

ATCC

Problem 2: Cryopreservation injury

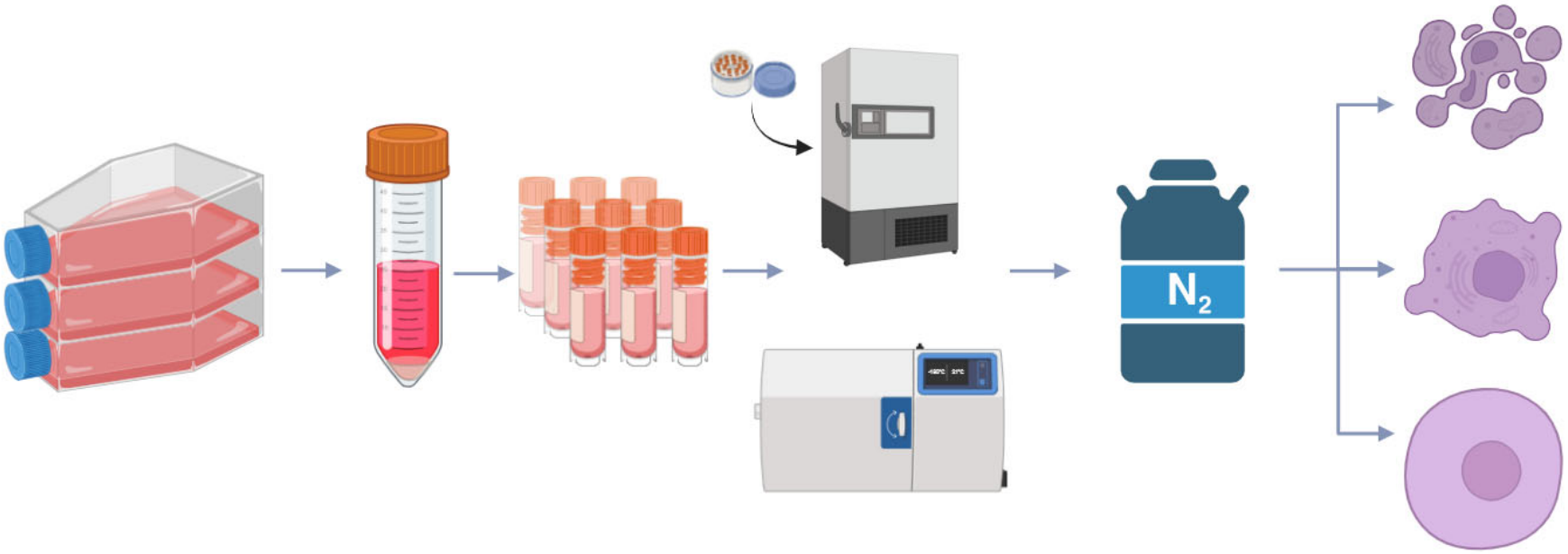


	Mechanism	Result
Osmotic Intolerance	▪ Water nucleates outside the cell, extracellular ice forms ▪ Cells and solutes concentrate in the inter-crystal space ▪ Water is drawn out of the cell to equilibrate osmotic pressure	▪ Osmotic stress can irreversibly damage cell membranes ▪ Cells unable to regulate water content properly can undergo membrane lysis and cell death
Intracellular Ice Formation (IIF)	▪ Cells cooled too quickly cannot sufficiently dehydrate and ice crystals can form within the cell cytoplasm	▪ Ice crystals can damage intracellular structures, puncture membranes, and disrupt normal biochemical processes
Chilling Injury	▪ Cells are exposed to low, non-freezing temperatures ▪ Lipid bilayer becomes more rigid ▪ Metabolic imbalance due to temperature-dependent reactions	▪ Altered membrane permeability and loss of homeostasis can lead to irreversible damage and activation of apoptosis mechanisms
Cryoprotectant Toxicity	▪ Certain cryoprotectants can easily penetrate cell membranes ▪ Disruption of membrane integrity, cell metabolism, and DNA packaging	▪ Can cause cell stress and death if not properly managed ▪ Cryoprotectant concentration and exposure times must be meticulously managed

Created with BioRender.com

ATCC

Problem 2: Cryopreservation recovery



Harvest cells, suspend in CPA, aliquot into vials

Cryopreservation and storage

Thaw and cellular outcome

Created with BioRender.com

A majority of cells survive cryopreservation but require post-thaw recovery in culture due to cellular injury

The solution: Assay ready?

Varying industry definitions (Assay ready? Ready-to-assay? Ready-to-plate? Thaw-n-go?)

Key characteristic: Save time, reduce cost, promote consistency

Assay Ready (Assay Preparation)

- Generally applicable and platform agnostic
- **Pre-plated** frozen solutions fall under this category. Face challenges related to storage stability
- Formats designed to replace cell banks – Lack authentication and proof of assay readiness

Assay Ready (Engineered & Kits)

- Generally **kitted solution** offering optimized companion reagents and other cells.
- Reporter **engineered cells** that readout within the confined assay.
- Optimized for a **single assay type** and single assay readout.
- Lack versatility – may be difficult to find solution that fits specific need

Assay Ready (Custom Banks)

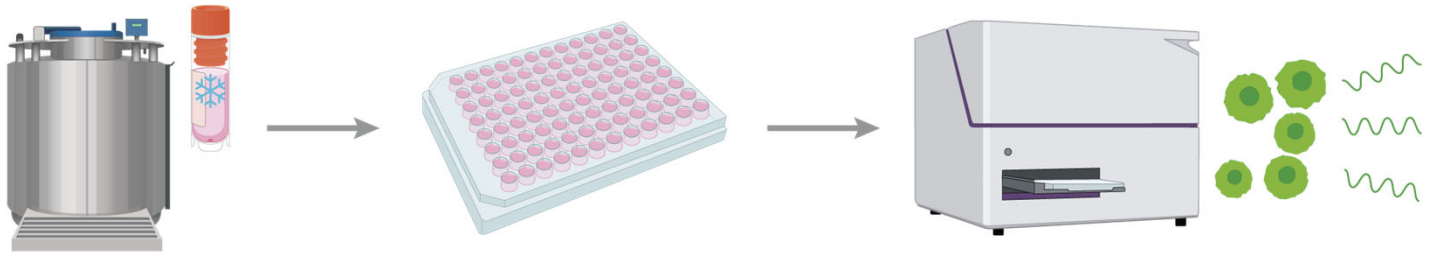
- Seed stocks / working cell stocks / **cell banks** created for direct use in assays.
- Mostly **custom sourced** with exact specifications.
- Assay readiness usually linked to **high cell density** in vials.
- Still **requires cell recovery** and assay validation.
- Not really an assay ready solution.

ATCC

ATCC's Assay Ready solution

From frozen to data in 1 day

ATCC Assay Ready Workflow (Hours)



1. Consumable vial format
   - Substitute for cell banks with vials quality tested for low intra- and inter-lot variability
   - Minimal change to existing bioassay workflows
2. Versatility
   - Input cells into a variety of assay workflows
   - Provide a catalog of application data
3. Same ATCC quality with improved specifications
4. Unique cryobiology
   - Dedicated team of experts to develop an optimized cryopreservation medium and process that enhances recovery
   - Assay Ready Cells can be plugged into assay workflows immediately post-thaw

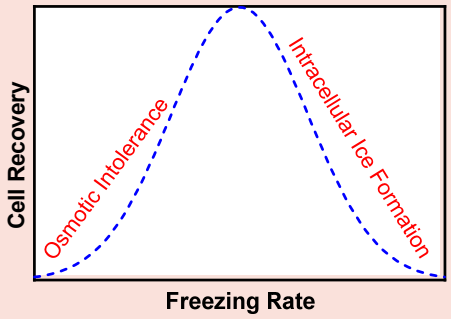
Cryopreservation optimization

Cryomedia Formulation (Factor 1)

Penetrating CPAs
- DMSO, glycerol
- Prevent intracellular ice formation
- Toxic

Non-penetrating CPAs
- Sugars, proteins, polymers
- "Additives"
- Protect membrane or modulate ice crystal morphology

Freezing Profile (Factor 2)



Controlled-rate freezer
- Finely tune rates and times
- Advanced freezing profiles

Assay Ready Formulation

- Cell specific
- Animal by-product free
- Low toxicity
- Optimized to enhance post-thaw recovery

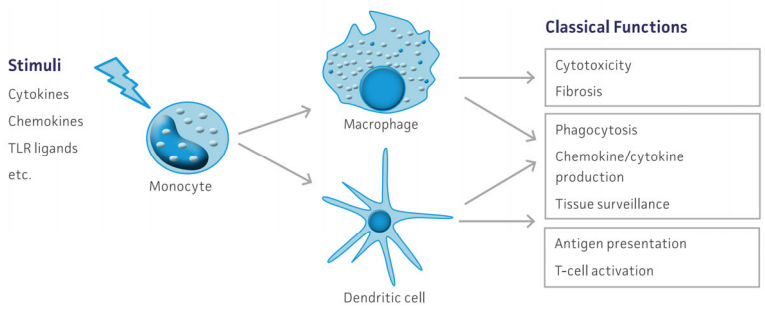
ATCC

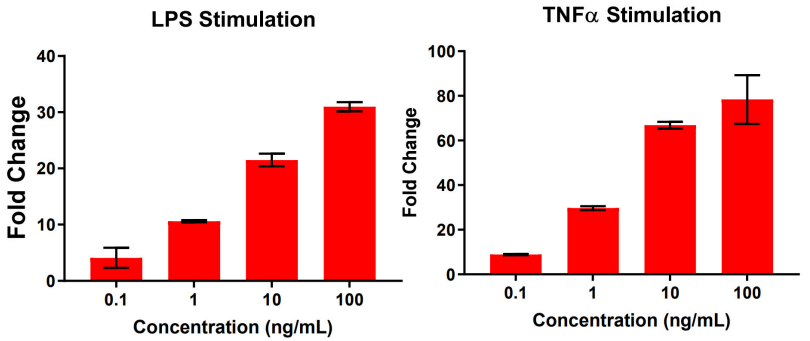
Application data

ATCC THP-1 cells & reporter

The first conversions

- **ATCC® TIB-202™**
  - THP-1 is a monocyte isolated from peripheral blood from an acute monocytic leukemia (ALL) patient.
  - Spontaneously immortalized.
  - Used to study immune function in vitro replacing expensive & variable human PBMC derived-monocytes and macrophages.
  - Monocyte/macrophage functions, mechanisms, signaling pathways, and nutrient and drug transport.
- **ATCC® TIB-202-NFkB-LUC2™**
  - Luciferase reporter cell line derived from the THP-1 parental line.
  - Expresses firefly luciferase gene (luc2) under control of a NF-κB promoter.
  - This reporter cell line is useful for monitoring the activity of IFN-γ induced signaling pathways.





From frozen to data in 1 day

Assay Ready cells meet/exceed all critical product attributes

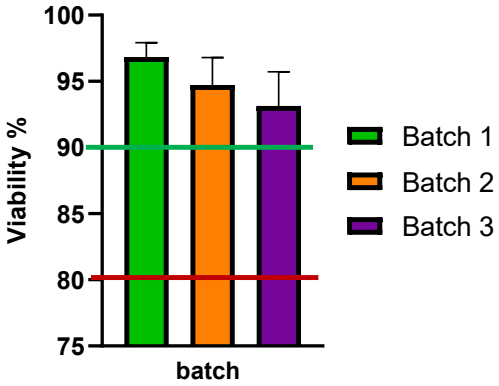
- High viability
- Low inter-/intra-lot variation/variability
- Growth and morphology similar to that of parental cells

Application data - assays complete in 1 work day

- Differentiation and morphology
- qPCR
- Flow cytometry
- Phagocytosis assay
- Luciferase assay

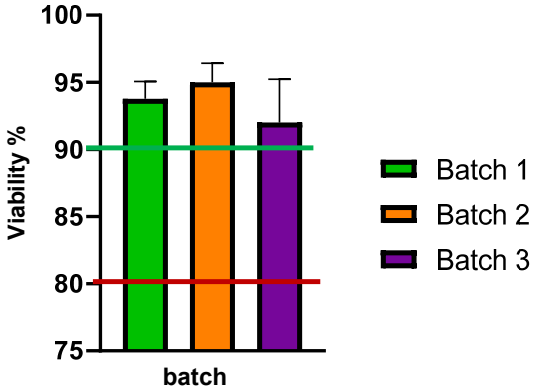
ATCC®

Assay Ready Cells: viability & consistency



Measured directly from frozen

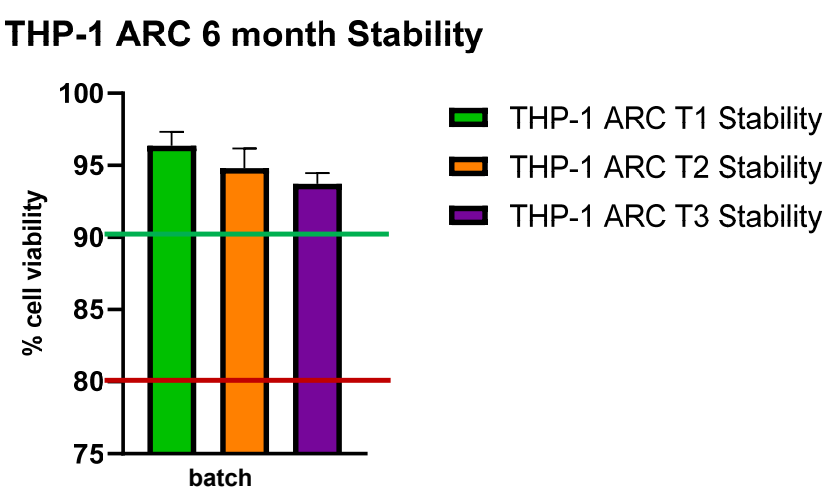
Also tested across multiple operators



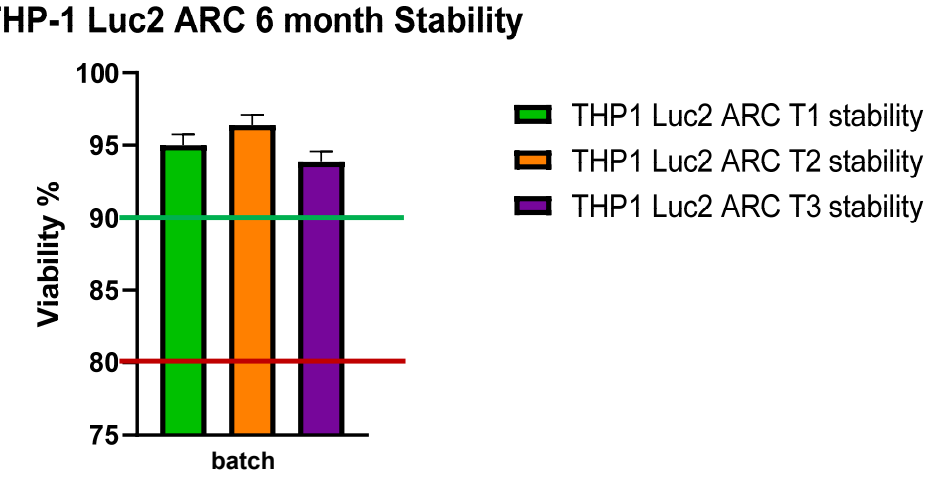
Post-thaw viability of THP-1 Assay Ready Cells: THP-1 Assay Ready Cells from three batches were thawed, and post-thaw viability was measured (Vi-CELL® BLU).

Post-thaw viability of THP-1 NFκB-Luc2 Assay Ready Cells: THP-1 NFκB-Luc2 Assay Ready Cells from three batches were thawed, and post-thaw viability was measured (Vi-CELL® BLU).

THP-1 Assay Ready Cells: 6-month stability



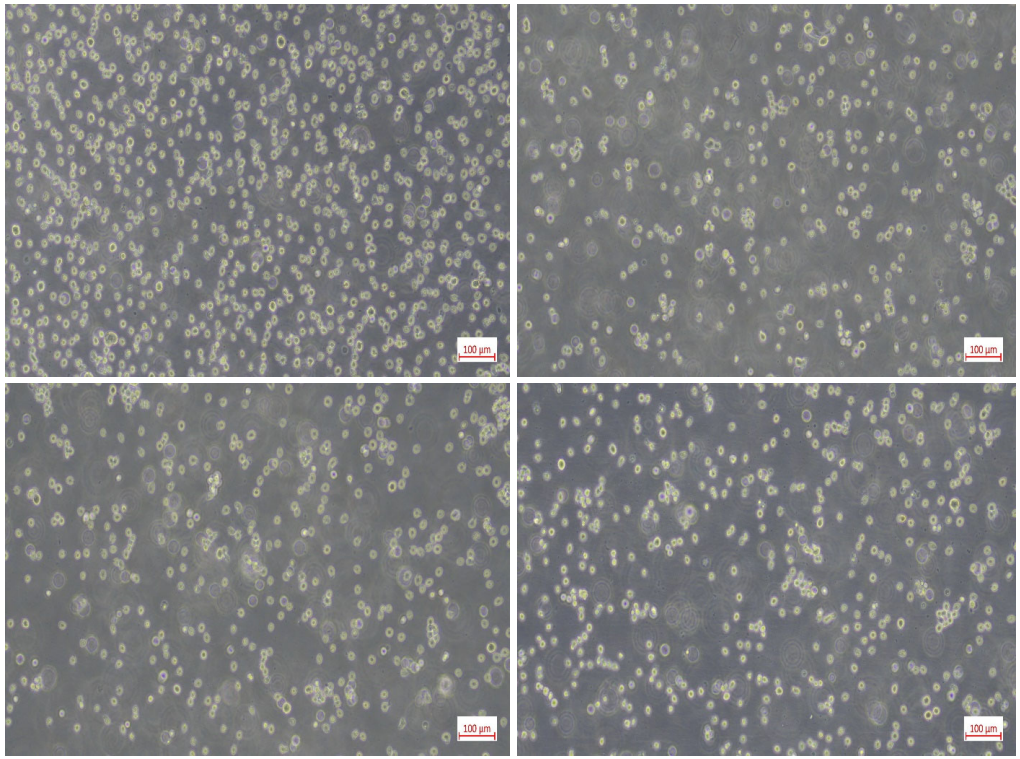
THP-1 Assay Ready Cells were stored in the vapor phase of liquid nitrogen for 6 months. Vials from three batches were thawed, and post-thaw viability was measured (Vi-CELL® BLU).



THP-1 NFκB-Luc2 Assay Ready Cells were stored in the vapor phase of liquid nitrogen for 6 months. Vials from three batches were thawed, and post-thaw viability was measured (Vi-CELL® BLU).

Assay Ready Cells exhibit morphology similar to that of parental cells

Live Control



Batch T1
24 hr post-thaw

Batch T2
24 hr post-thaw

Batch T3
24 hr post-thaw

Optimized companion protocols for Assay Ready Cells

Cell thawing:

ASSAY READY CELL THAWING PROTOCOL

BEFORE THAWING, PREPARE THE FOLLOWING:

- Labeled 15 mL tubes (1 per vial to be thawed)
- Complete medium, pre-warmed
- Just prior to thawing, aliquot 9 mL of warmed complete culture medium to each 15 mL tube

THAWING:

1. Retrieve the desired number of frozen vials from the appropriate LN2 freezer.
2. Quickly transport and place the vials in a floating mat in a 37°C water bath, immersing the vials up to the level of the cap. Thaw for approximately 3 min.
3. Remove all vials from the water bath at the same time and dry with a laboratory wipe. Spray vials with 70% ethanol or equivalent, while gently inverting the vials 2-3 times to mix the contents. Dry vials again with a laboratory wipe. All operations after this point should be carried out under aseptic conditions.
4. Transfer the vials to a biosafety cabinet and aseptically loosen the vial caps. Using P1000 tips, gently and slowly, transfer the cell contents, to the 15 mL sterile centrifuge tube(s) containing 9.0 mL complete growth medium. After transferring all cells, tighten the centrifuge tube caps and mix the cell suspension by gently inverting the centrifuge tube 2-3 times.
5. Centrifuge at 150 to 300 x g for 8 to 12 minutes at room temperature.
6. Aseptically loosen the caps of all centrifuge tubes, discard the supernatant by vacuum, taking care to not disturb the cell pellet.
7. Add 10 mL of pre-warmed complete growth media to each of the tubes. Gently resuspend cell pellet with a 10 mL disposable pipette by pipetting up and down for a few times.
8. Immediately remove an appropriate amount of cell suspension and transfer to a cell counting tube.
9. Transfer tube to your cell counting instrument. Count the cells.
10. Plate cells as appropriate for your assay.

ATCC

Optimized companion protocols for Assay Ready Cells

Differentiation to macrophage-like cells:

INTRODUCTION:

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

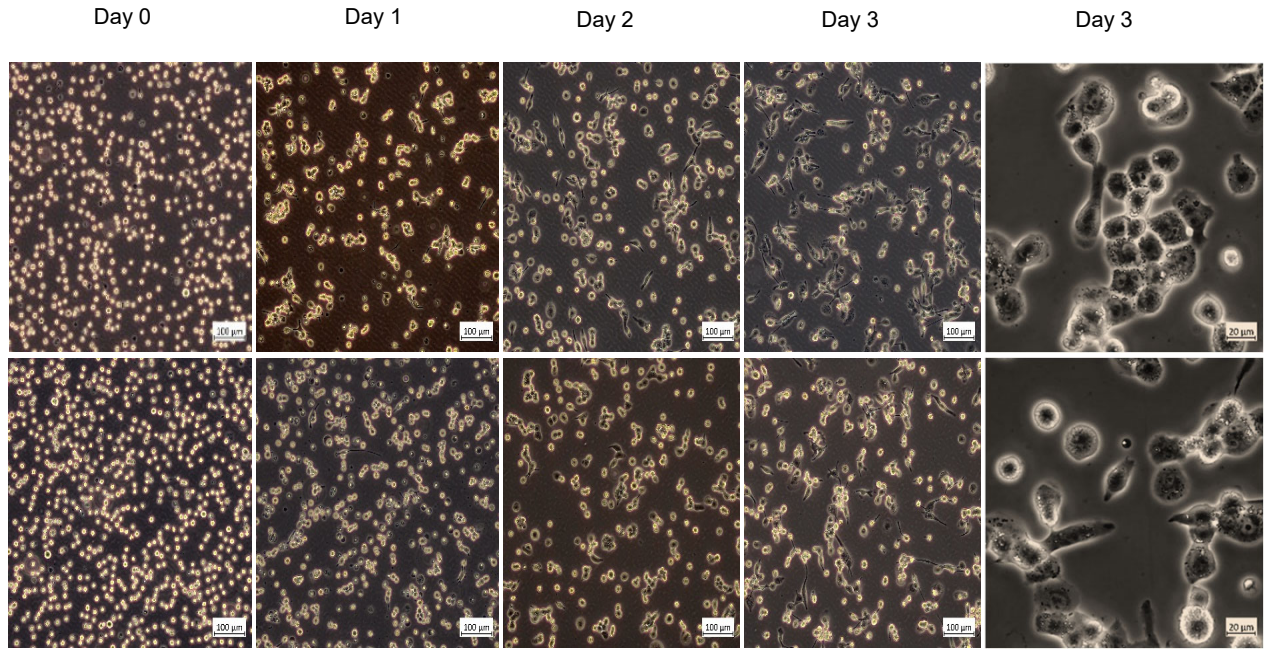
CELL SEEDING AND DIFFERENTIATION PROTOCOL:

4. **Seed cells:**
   - After thawing the Assay Ready Cells (follow ATCC recommended thawing procedure for Assay Ready 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

Assay Ready differentiation

Cell morphology during macrophage differentiation in THP-1 cells

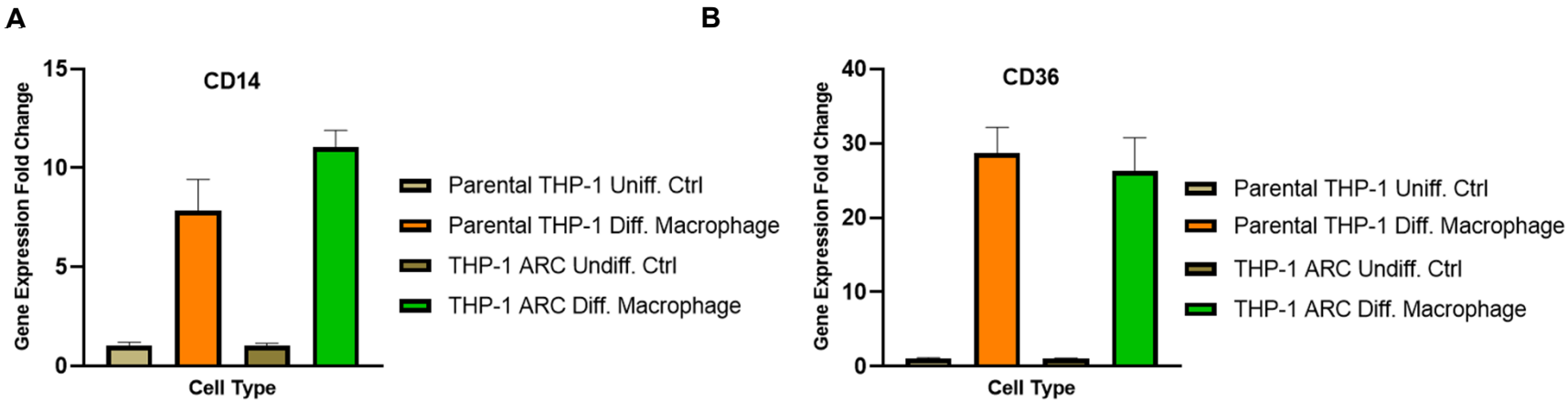


Assayed directly from frozen

Changes in Cell Morphology During PMA-Induced Macrophage Differentiation.
Parental THP-1 and freshly thawed THP1 Assay Ready cells were plated and treated with PMA for 72 hours to differentiate into macrophage-like cells. Cell morphology was observed under the microscopy and cell images were captured using a digital camera at 0, 1, 2, and 3 days after PMA stimulation.

THP-1 Assay Ready Cell differentiation: application assays

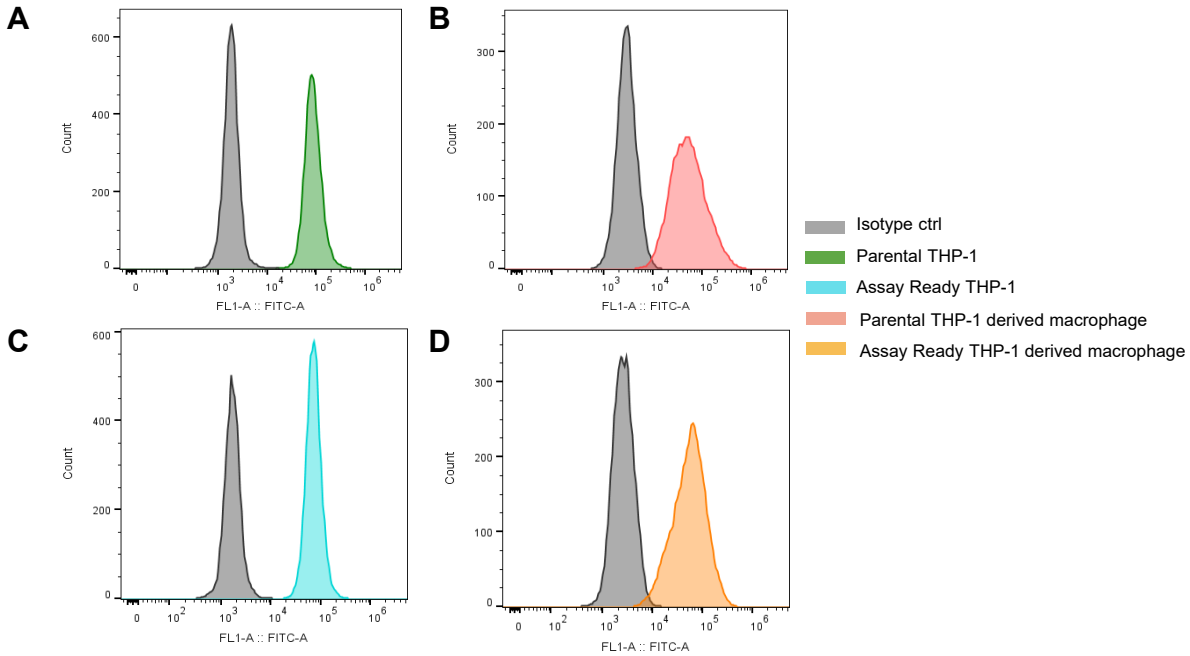
qPCR



Increased mRNA Expression of CD14, CD36 in PMA Induced Macrophage-like Cells.
Parental THP-1 and freshly thawed THP-1 Assay Ready cells (ARCs) were plated and treated with PMA for 72 hours 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 Assay Ready cells were increased compared to the undifferentiated controls.

THP-1 Assay Ready Cell differentiation: application assays

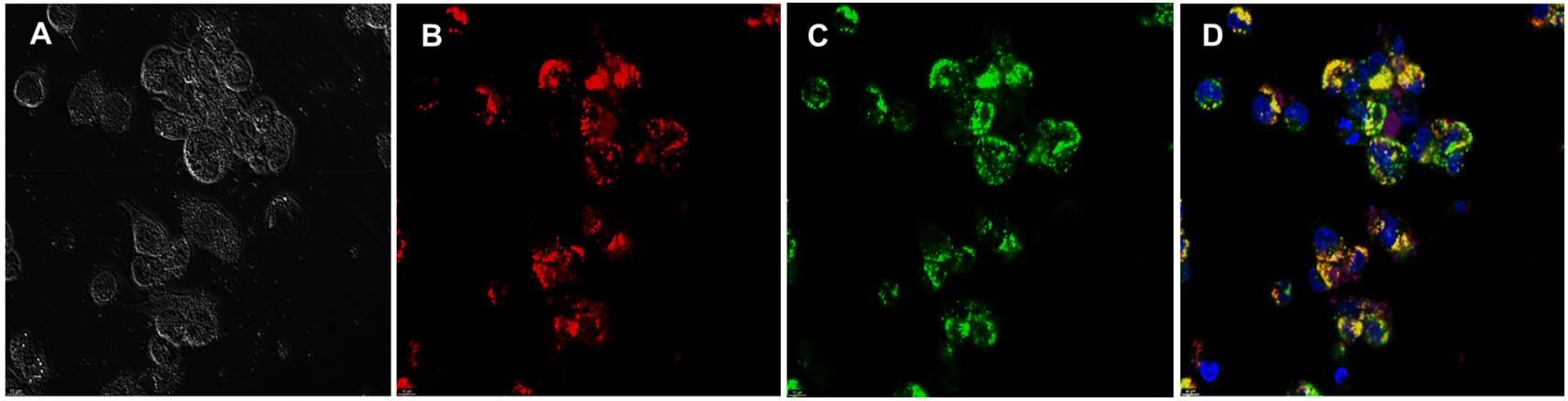
Flow cytometry



CD14 Surface Marker 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).

THP-1 Assay Ready Cell functional assays

Phagocytosis Assay

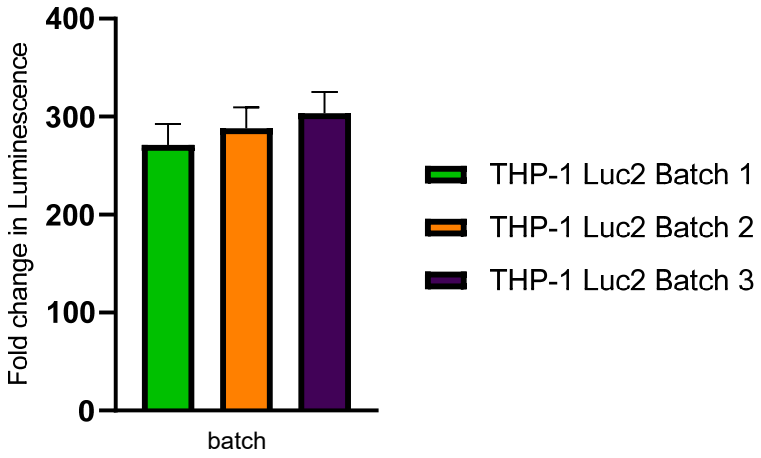


Phagocytosis assay of THP-1 ARC-derived macrophage-like cells.
Freshly thawed THP-1 Assay Ready cells were incubated with PMA for 3 days to differentiate into macrophage-like cells. (A) Phase contrast image of the differentiated macrophage-like cells. (B) Cells undergoing phagocytosis with ingested pHrodo™ (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 the phagocytosis. DAPI (Thermo Fisher Scientific®) stained nuclei showed in blue.

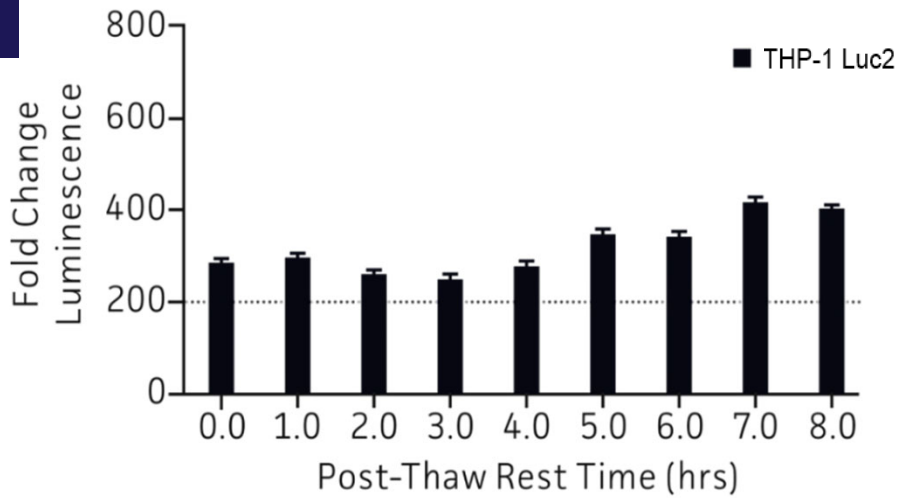
THP-1 NFkB-Luc2 Assay Ready Cell application data

Application data – Luciferase induction after LPS challenge

Assayed directly from frozen



Post-thaw luciferase expression in THP-1 NFκB-Luc2 Assay Ready Cells after LPS stimulation (50 ng/mL).

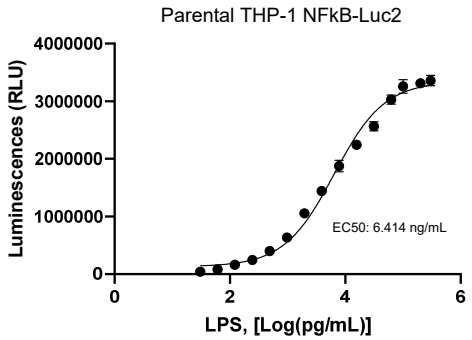


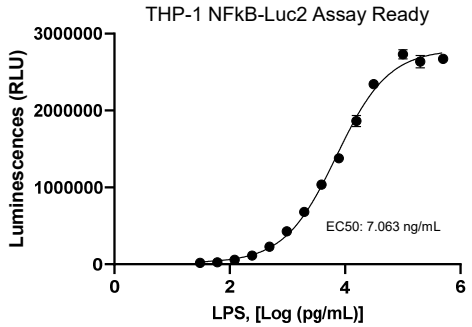
Post-thaw rest time (0-8 hrs) luciferase expression in THP-1 NFκB-Luc2 Assay Ready Cells after LPS stimulation.

THP-1 NFkB-Luc2 Assay Ready Cell application data

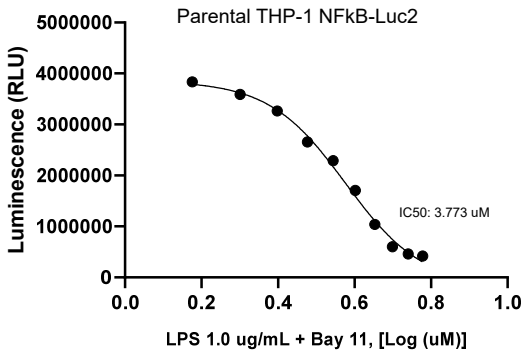
Dose-dependent response curves

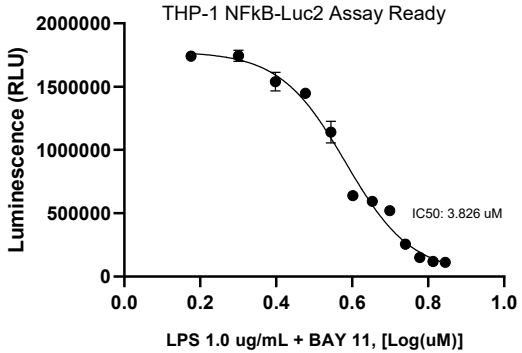
A





B





Bioluminescence signal intensity dose-dependence response curve to compounds treatment.
THP-1 NFkB-Luc2 parental cells and post-thaw THP-1 NFκB-Luc2 Assay Ready Cells were seeded in the wells of 96-well plates. After 2 hours recovery, cells were treated with (A) a series of concentrations of LPS, or (B) 1.0 ug/mL of LPS, and a series of concentrations of BAY-11. After 3 hours stimulation, bioluminescence signal of the cells was detected using Bright-glo™ (Promega®) and a luminometer (Glomax®).

Data Summary

- Cell expansion has been optimized by ATCC and cells have been frozen with a proprietary ABP-free cryomedia
- Assay Ready Cells provide high post thaw viability and cell count, as well as authenticated cells for reliability/repeatability
- THP-1-AR and THP1-Luc2-AR cells have been extensively tested in application assays and show the appropriate marker expression and functionality
  - ATCC® **TIB-202-AR™** cells can be differentiated into macrophage-like cells (protocol provided)
    - Cells exhibit hallmark morphological characteristics of macrophages
    - Express increased levels of CD14 and CD36 upon PMA activation
    - Cells retain phagocytic capabilities
  - ATCC® **TIB-202-NFκB-LUC2-AR™** cells
    - Robust luciferase expression upon LPS stimulation
    - Appropriate drug response curves

Summary

- ATCC's Assay Ready Cells are a direct substitute for cell banks and can be input into bioassay workflows immediately post-thaw
- Please visit attc.org for more information on our assay ready cells
- Provide feedback after the presentation regarding what cells lines your lab would like to see considered for addition to the portfolio

ATCC: The Global Bioresource Center | ATCC

ATCC

Learn more: www.atcc.org/assayready

SITC Annual Meeting 2024

- George R. Brown Convention Center, Houston, TX
- Nov 8 – Nov 9, 2024, **booth #823**

Society for Laboratory Automation and Screening 2025

- San Diego Convention Center
- Jan 25 – Jan 29, 2025, **booth #2652**