Advancements in Human Cell Line Cryopreservation for Assay Ready Efficiency

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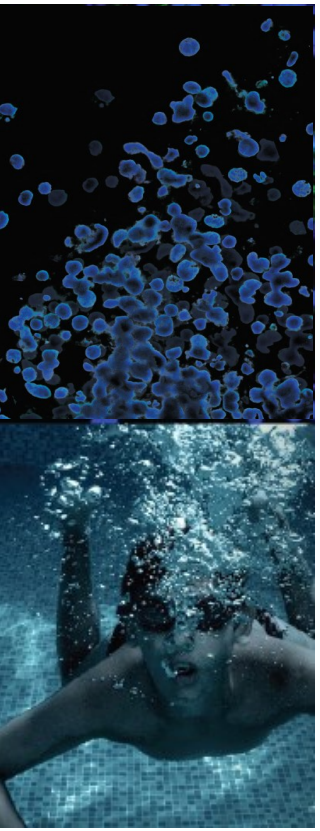
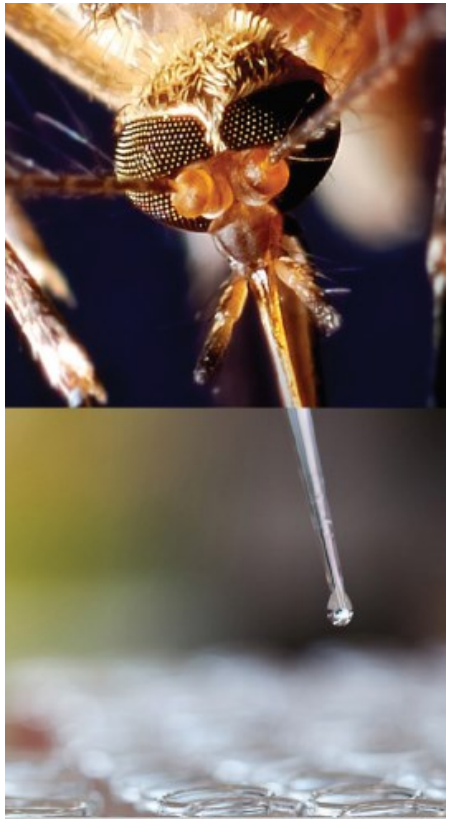
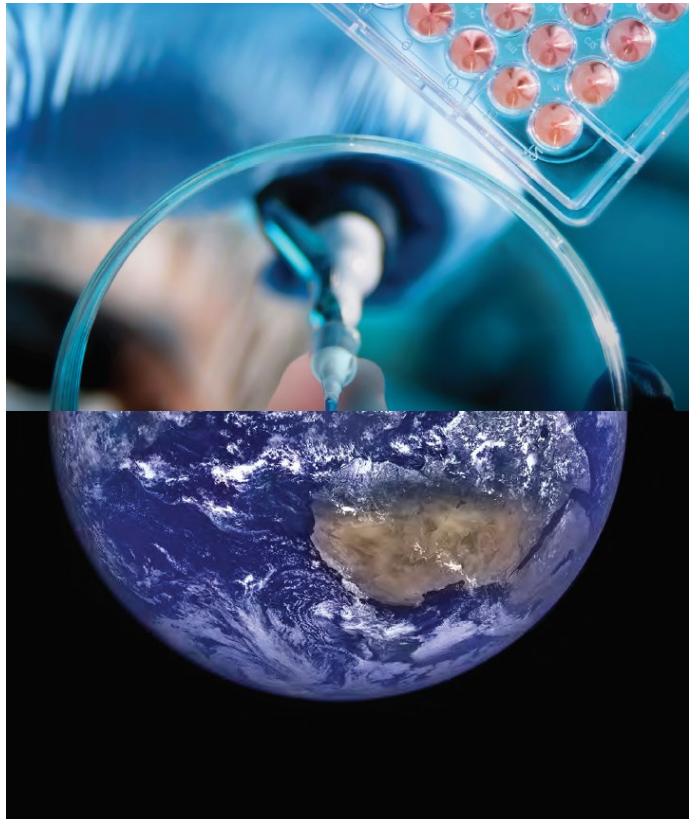


Credible Leads to Incredible™



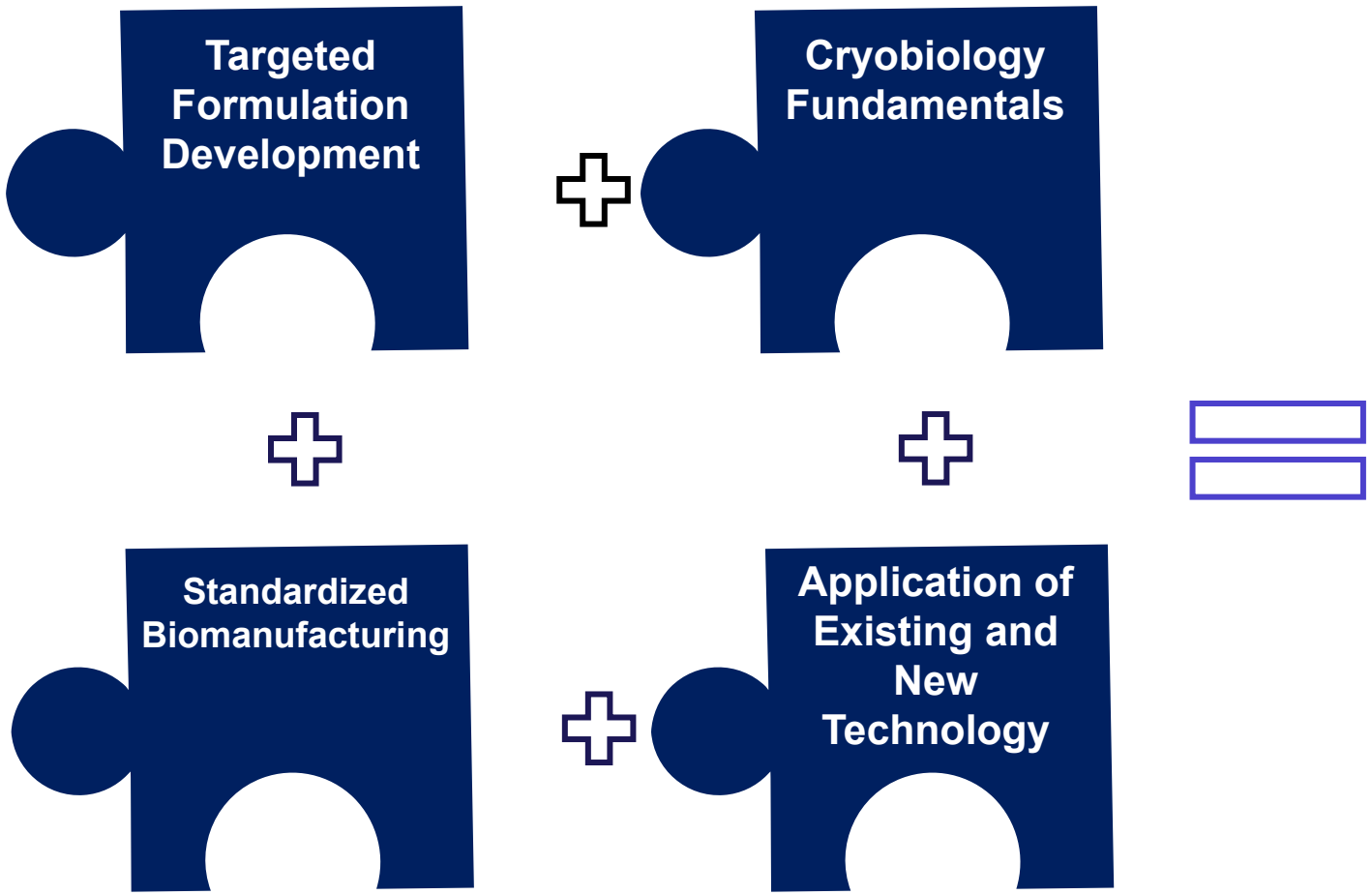


About ATCC®

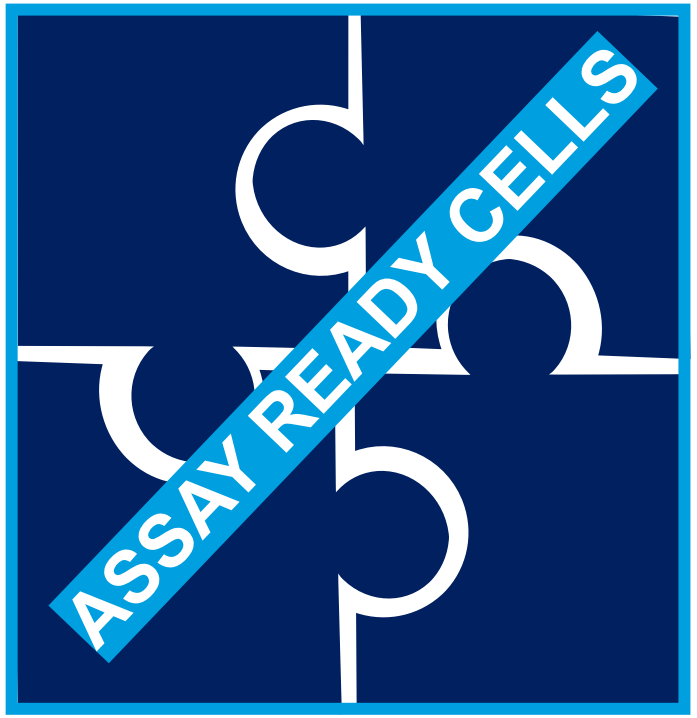
- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - 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, 19 international distributors
- Talented team of 500+ employees, over one-third with advanced degrees

Let's set the stage



New Product Format



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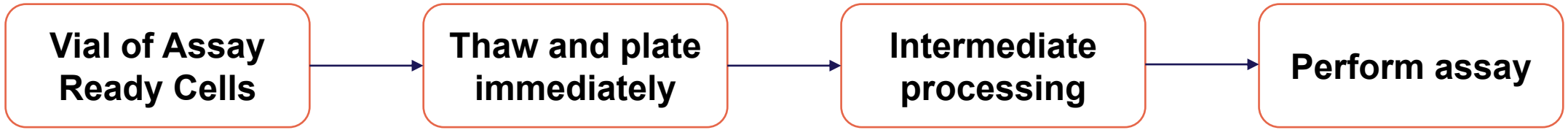
What is the Assay Ready format?

Market pain point

- Cell culture is time, resource, and labor expensive
- Long-term culture may experience sterility issues or phenotypic drift

Assay Ready Solution



Characteristics

- Remove requirement for continuous culture
- Rigorous quality validation
- Versatile assay applications

Saving time with Assay Ready cells

Assay development and pilot testing

Conventional

- Labs may be assessing feasibility of multiple cell models
- Short culture requirements for each can add up

Assay Ready

- “Go or no-go” decisions can be made faster

Generation of Working Cell Banks (WCBs)

Conventional

- Thaw and expansion of material can take 4-8 weeks
- Difficult to maintain consistency
- Large amount of resources

Assay Ready

- Assay Ready cells replace working cell bank
- Series of costs and challenges into a single product price point

Day-to-day Assay Performance

Conventional

- Vials thawed from WCB my take 1-2 weeks for full functional recovery
- More effort scheduling effort

Assay Ready

- Remove recovery culture, simplify scheduling.

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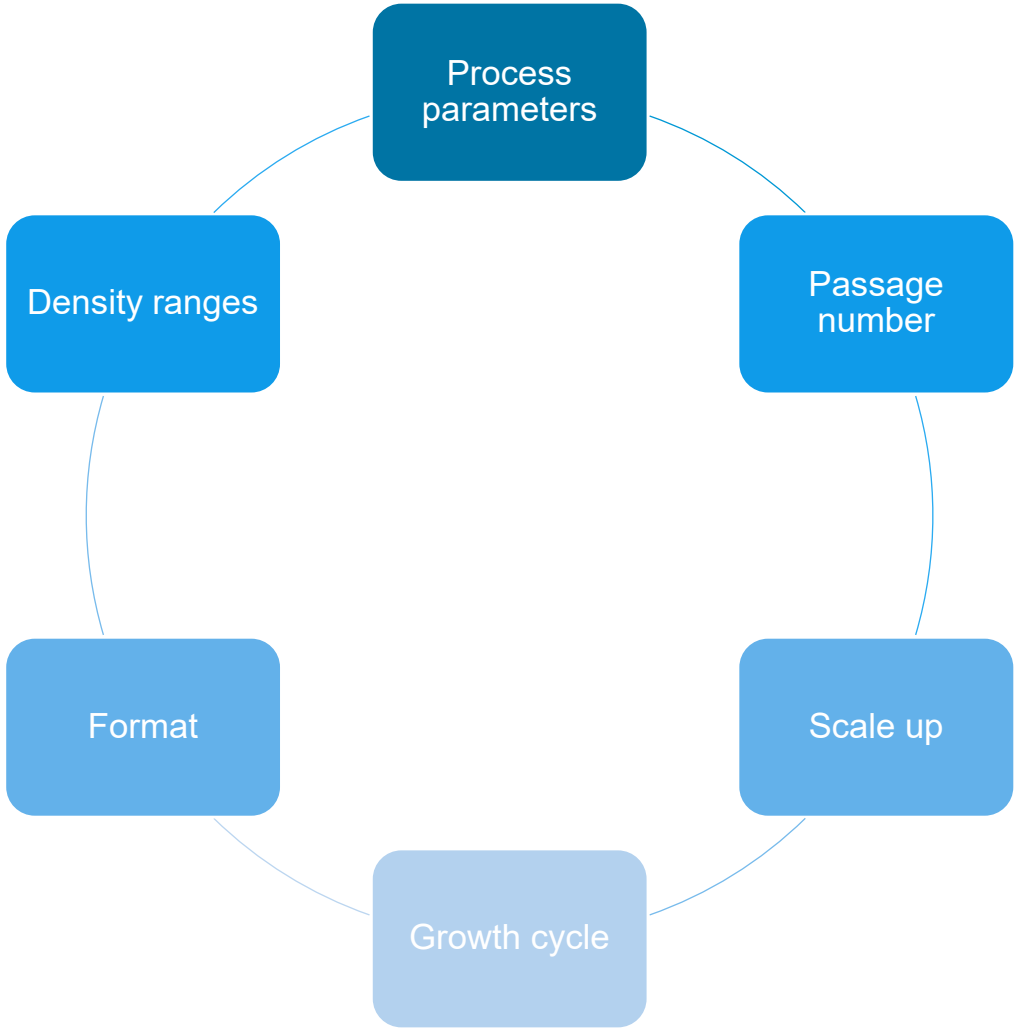
Ideation to realization

Maintaining market and product requirements through the series of processes enabling biological products

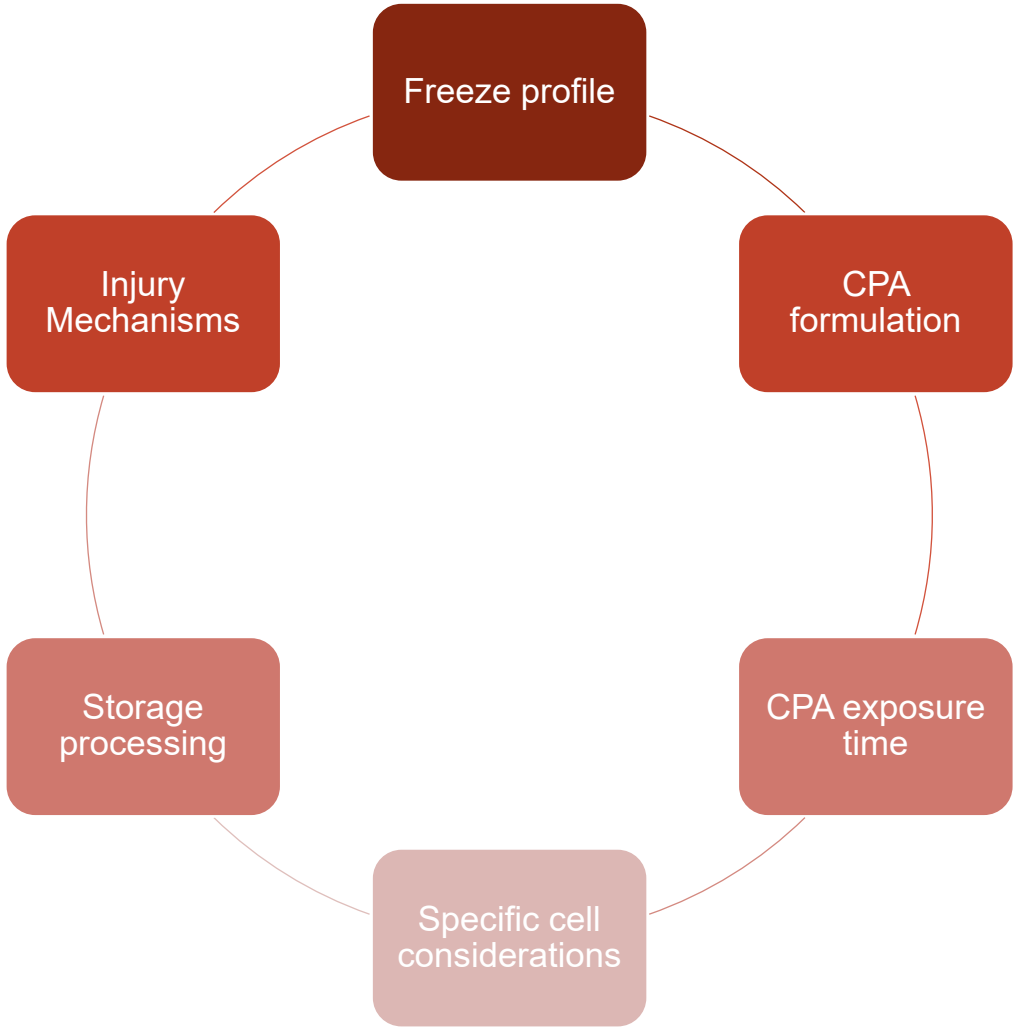
Bioproduction



Primary Goal: Consistency

- Strictly controlled set of process parameters for consistent phenotype at time of harvest
- Challenge: maintaining consistency through scale-up
- ATCC has decades of experience

Cryopreservation



Primary Goal: Maintain cell health and reduce recovery time

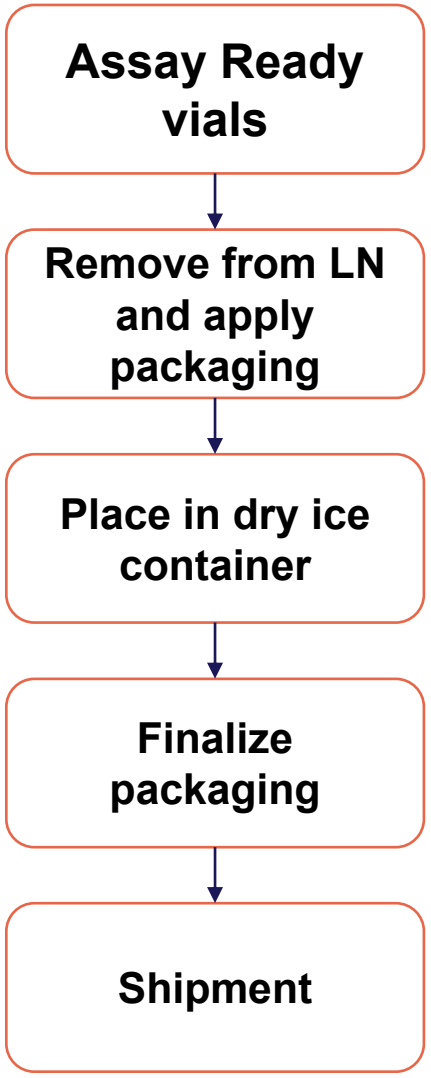
Secondary Goal: Standardization

Challenge: ice crystal injury and CPA toxicity with longer exposure times

- Proprietary cryoformulation
  - Animal by-product free (consistency)
  - Low toxicity
- Freezing process
  - Utilize validated controlled rate freezers for large scale cryopreservation
  - Automated vialing to reduce CPA exposure time
  - Proprietary freezing protocol to precisely control ice nucleation and propagation

Shipping

Packaging Workflow

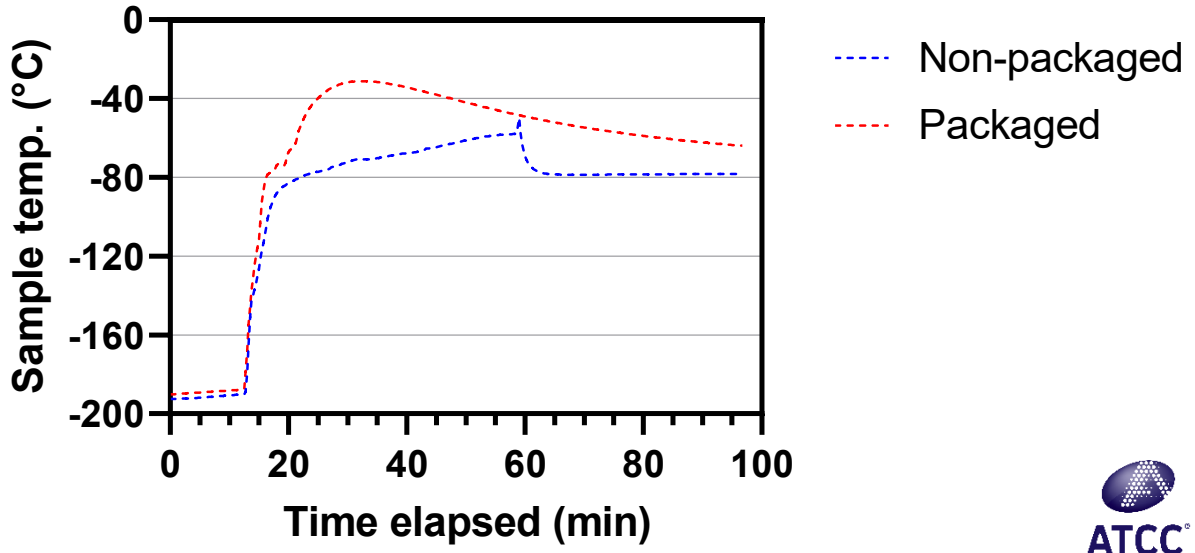


Primary Goal: Maintaining preservation stability

Challenges:

- Non-cryogenic shipping (dry ice)
- Complex packaging set-up
  - Primary receptacle
  - Secondary packaging
  - Outer packaging

Temperature probe of cryopreserved vials packaged and placed on dry ice



Application Data

Pilot Assay Ready products

THP-1-AR (ATCC® TIB-202-AR™) and THP-1-NFκB-Luc2-AR (ATCC® TIB-202-NFkB-LUC2-AR™)

- Assay Ready suspension cell
- Model for human monocytes and macrophages
- Data highlighting use in inflammatory studies, macrophage differentiation, and phagocytosis assays

Assessment of Assay Ready Efficacy

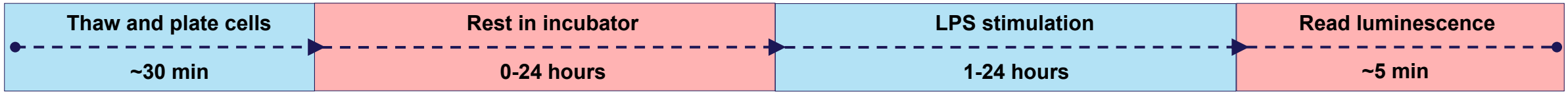
Assay Ready cells plated immediately post-thaw

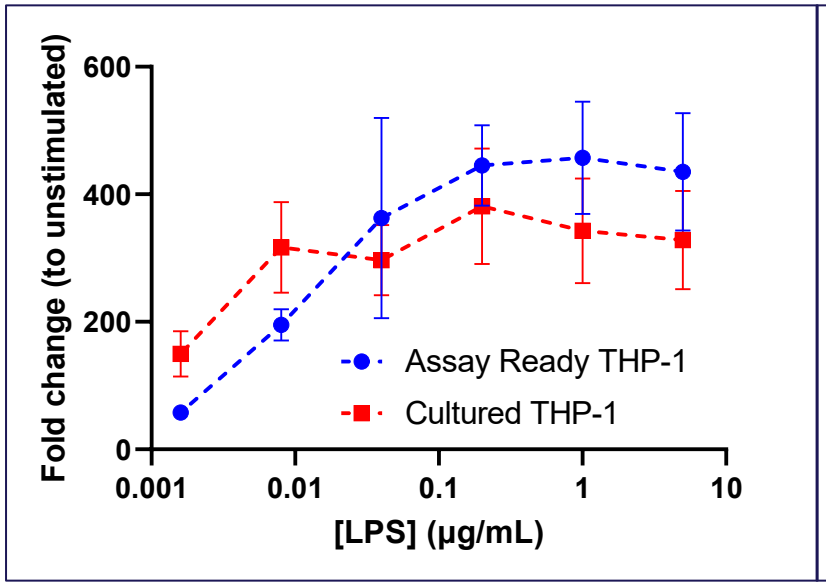
VS

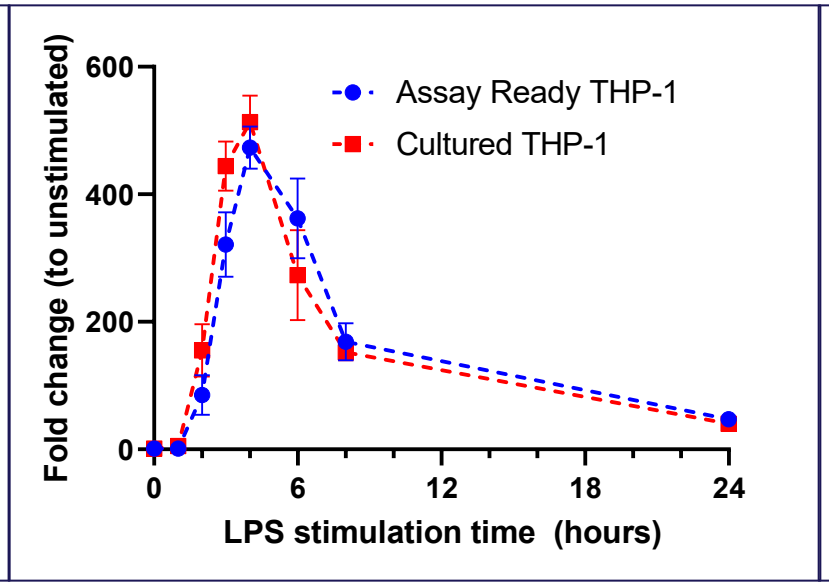
Propagation model plated from continuous culture

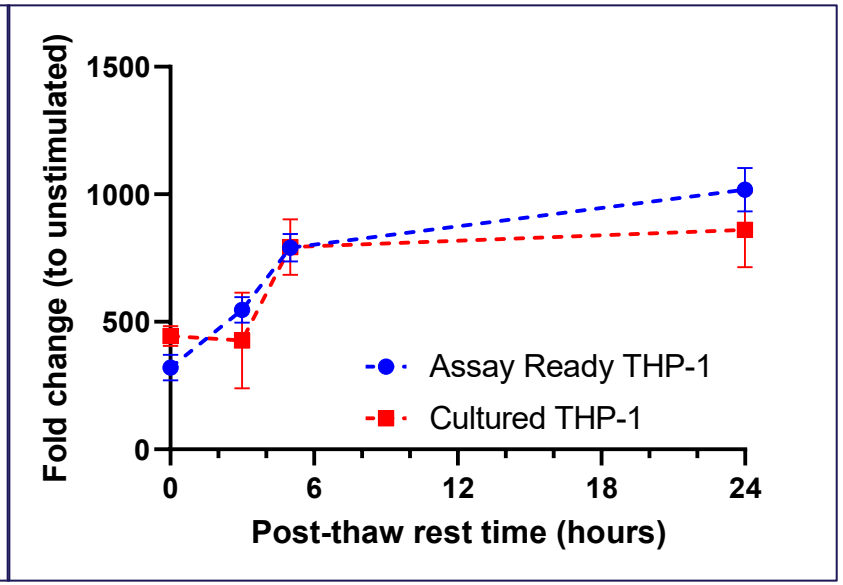
Inflammatory signaling – NFκB activation

THP-1-NFκB-Luc2-AR (ATCC® TIB-202-NFkB-LUC2-AR™) vs. Cultured THP-1-NFκB-Luc2 (ATCC® TIB-202-NFkB-LUC2™)



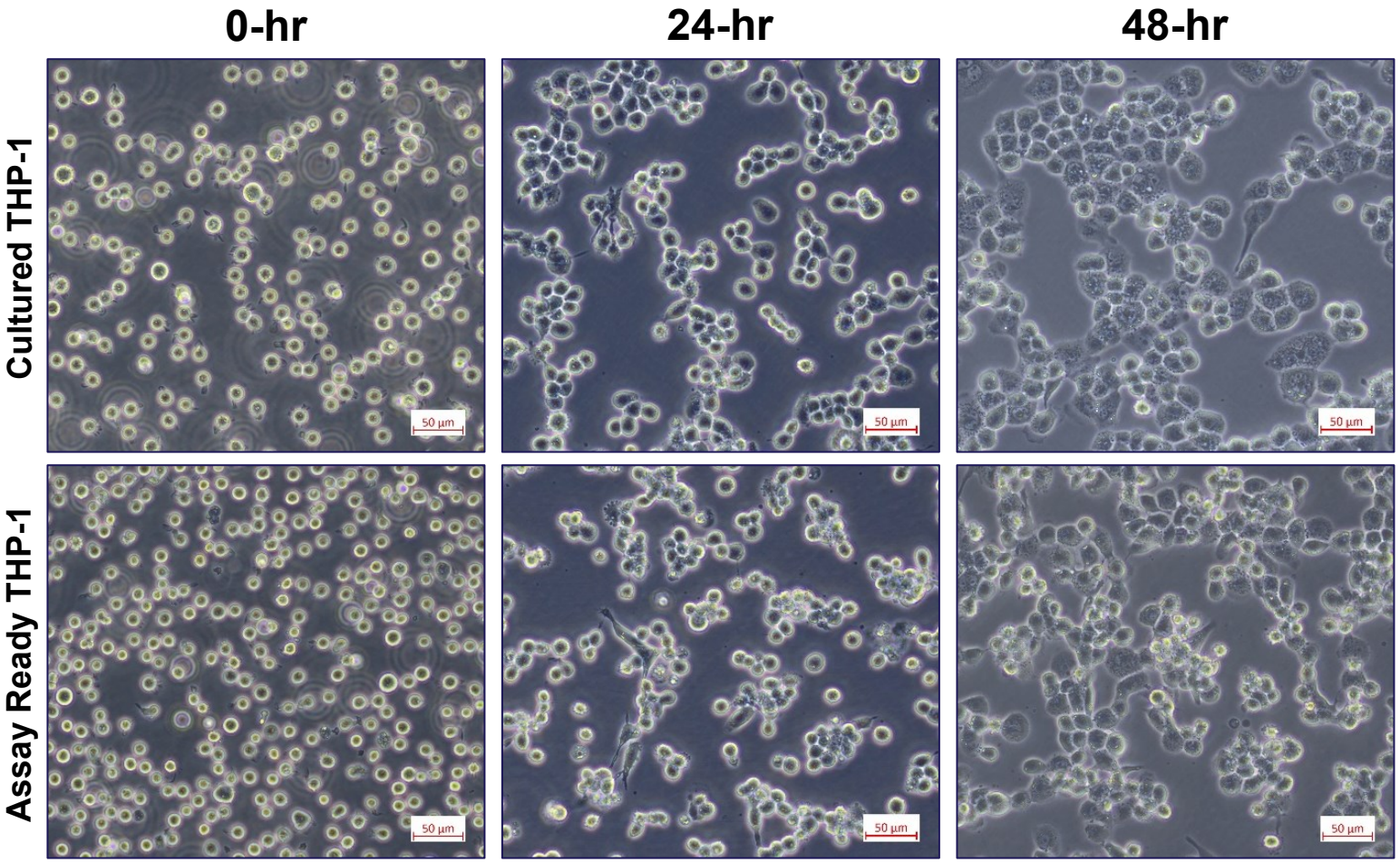






Macrophage differentiation: Morphology

THP-1-AR (ATCC® TIB-202-AR™) vs. Cultured THP-1 (ATCC® TIB-202™)



Morphology indicators of differentiation

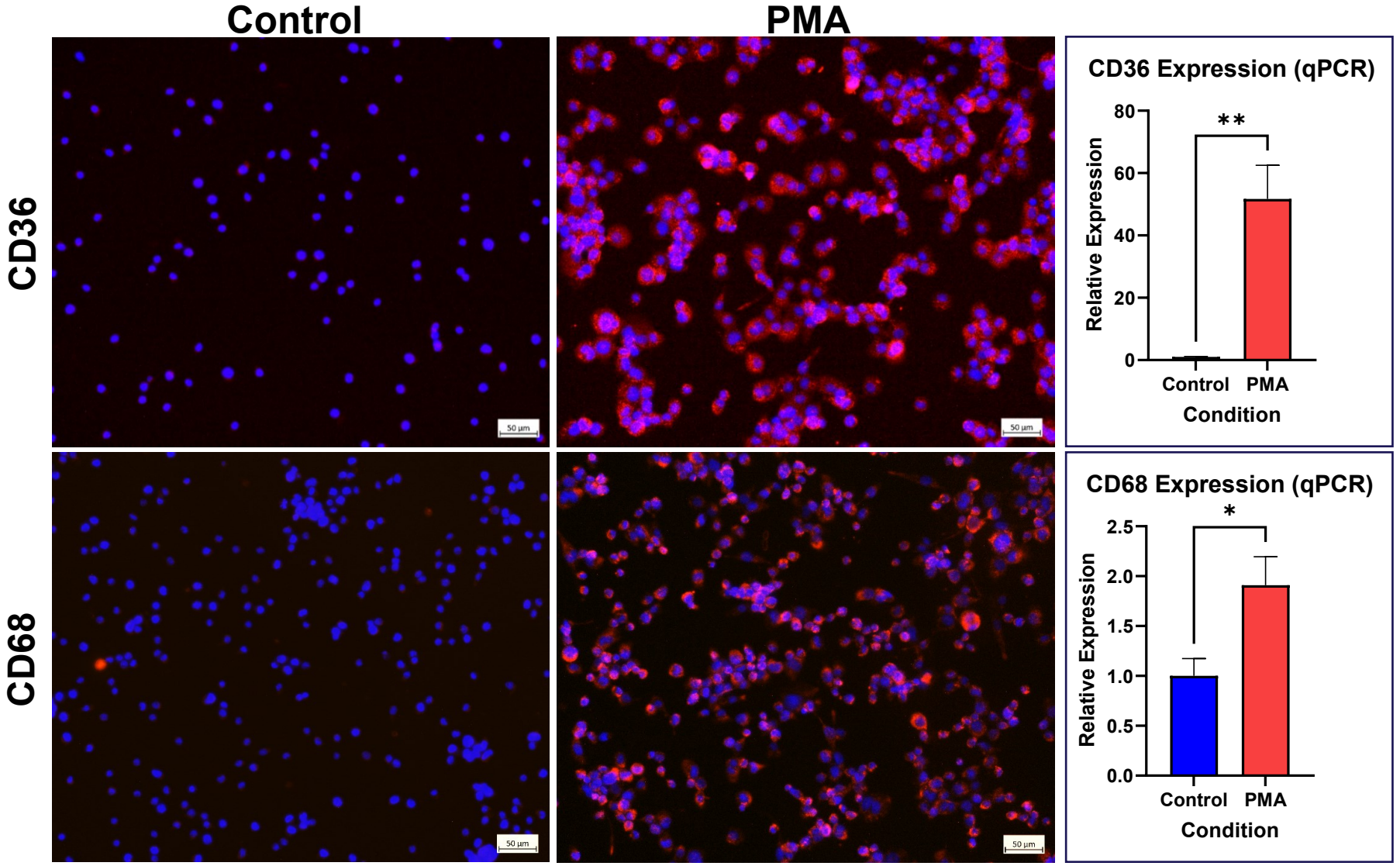
- Plate adherence
- Increase in cytoplasmic volume
- Enhanced granularity

Differentiation protocol

- 48 hours incubation
- 100 ng/mL phorbol 12-myristate 13-acetate (PMA)

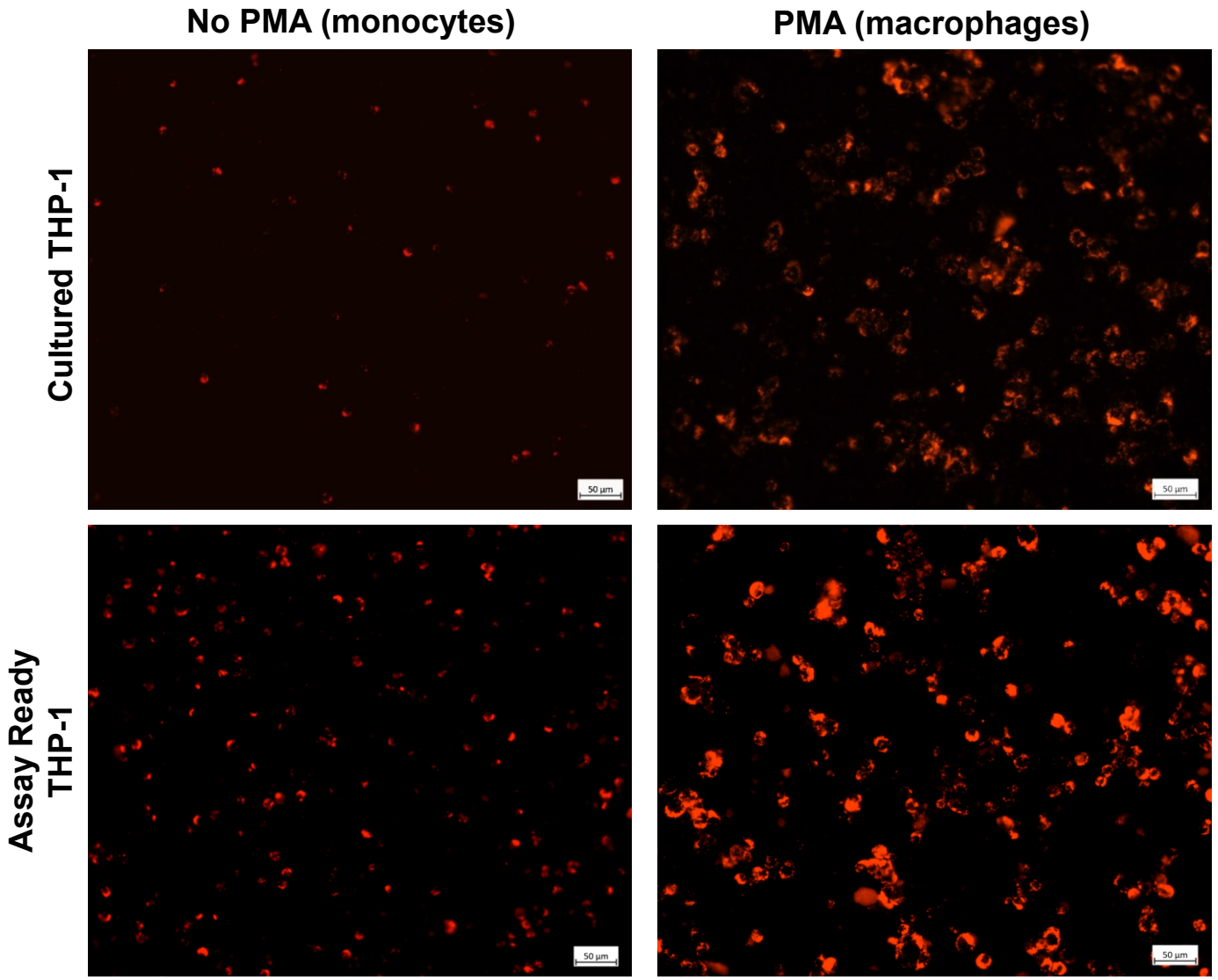
Macrophage differentiation: Marker expression

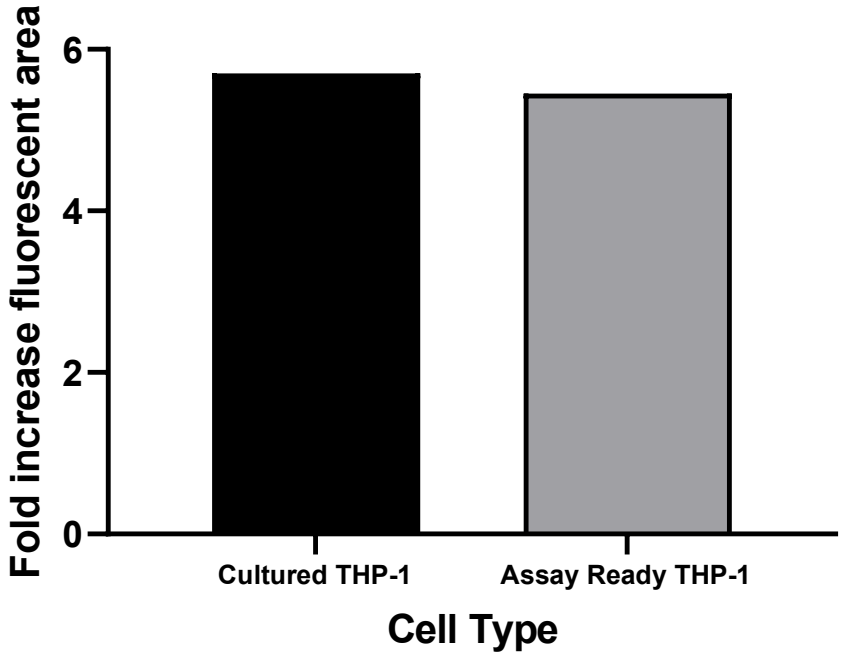
THP-1-AR (ATCC® TIB-202-AR™)



Phagocytosis (pHrodo)

THP-1-AR (ATCC® TIB-202-AR™) vs. Cultured THP-1 (ATCC® TIB-202™)

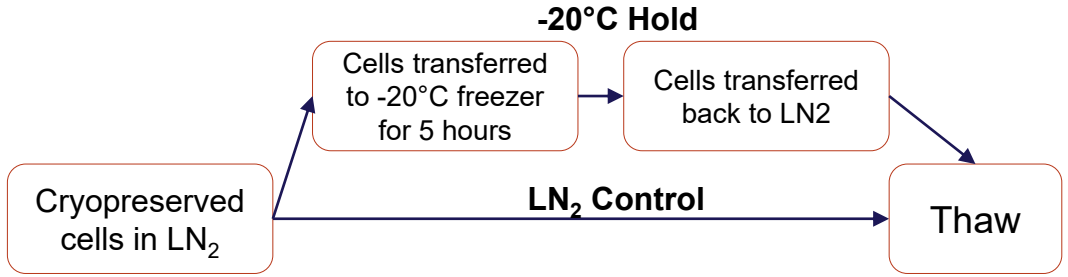


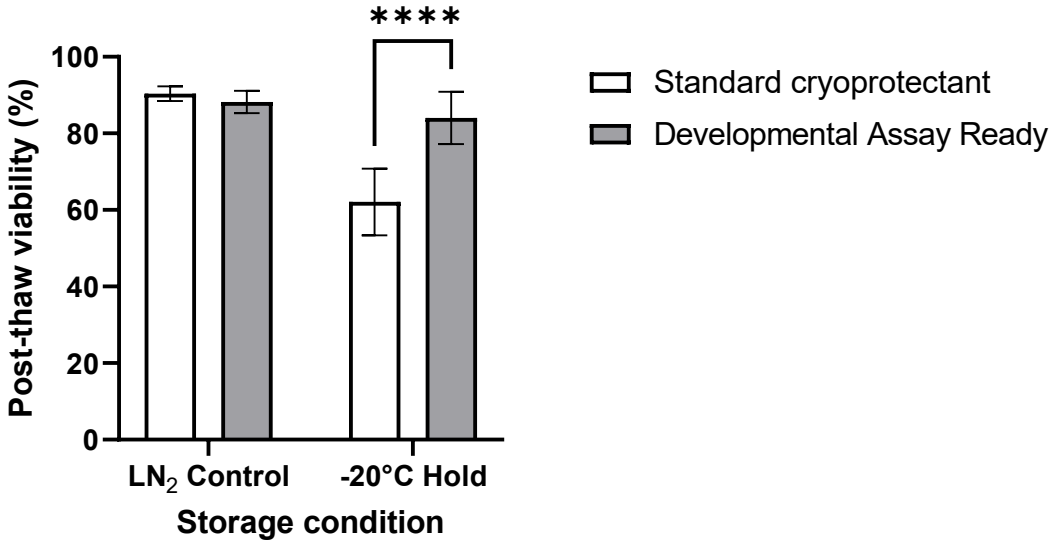


Hep G2

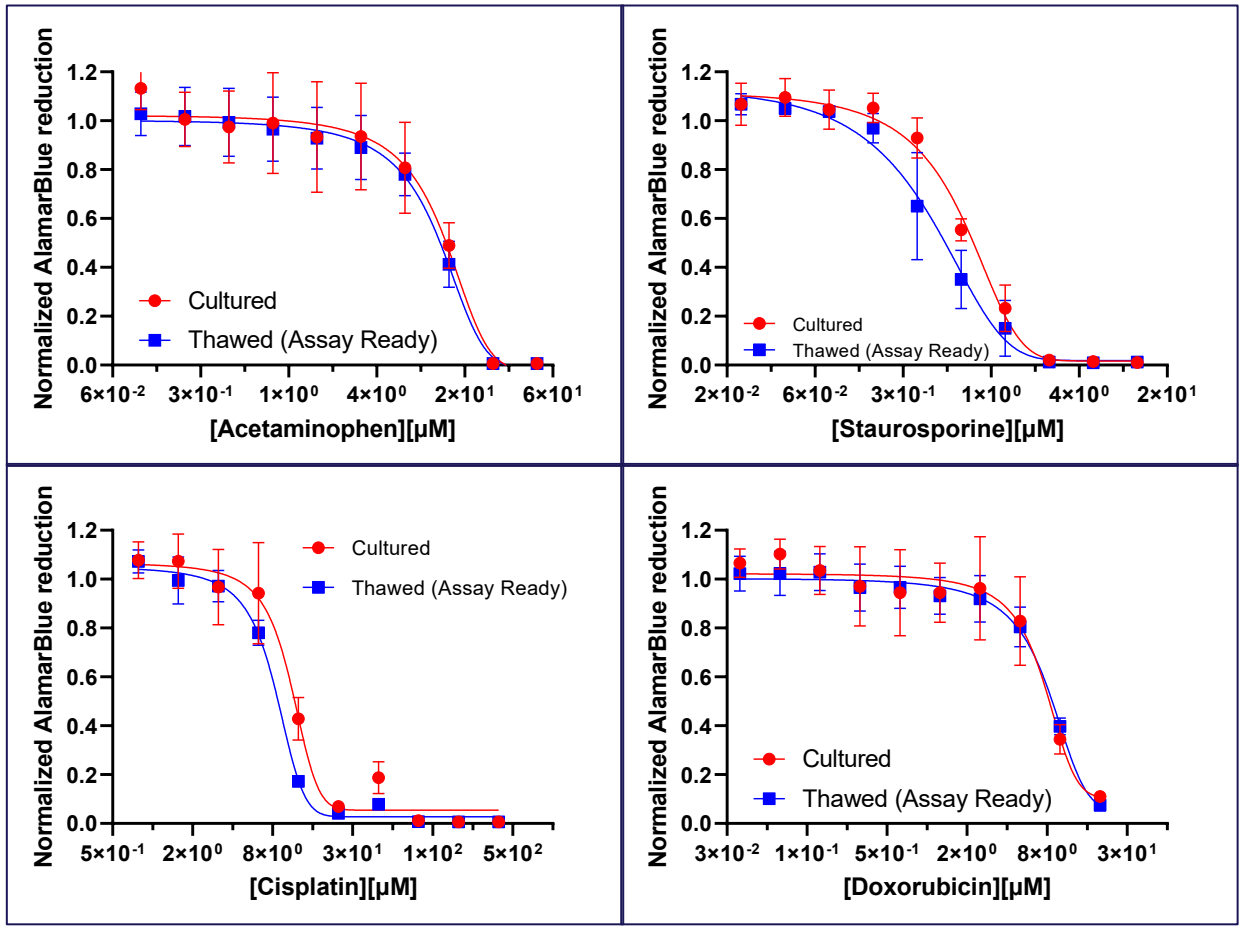
Adherent format – under development

CPA improves post-thaw outcome under simulated loss of temperature control





Drug toxicity response



Wrap-up

- Defined market pain point (continuous cell culture) and how the Assay Ready cells address it
- Moving from ideation to realization through market and product requirements and maintaining them through processes required for the biological format
- Introduced pilot products (THP-1-AR and THP-1-NFκB-Luc2-AR)
  - Pro-inflammatory pathways (NFκB)
  - Macrophage differentiation (morphology, markers)
  - phagocytic uptake tracking
- Hep G2 developmental data
  - New formulation enhancing shipping stability
  - Plugging into drug toxicity response

Acknowledgments

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Thank you!