From Curiosity to Breakthroughs:
Accelerate Your Drug Development with Assay Ready Cells

Fatah Kashanchi, PhD, Professor, George Mason University
Heather Branscome, PhD, Senior Scientist, ATCC

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 largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring new product formats that support drug development, safety testing, and high-throughput screening
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

bsi. ISO 9001 Quality Management

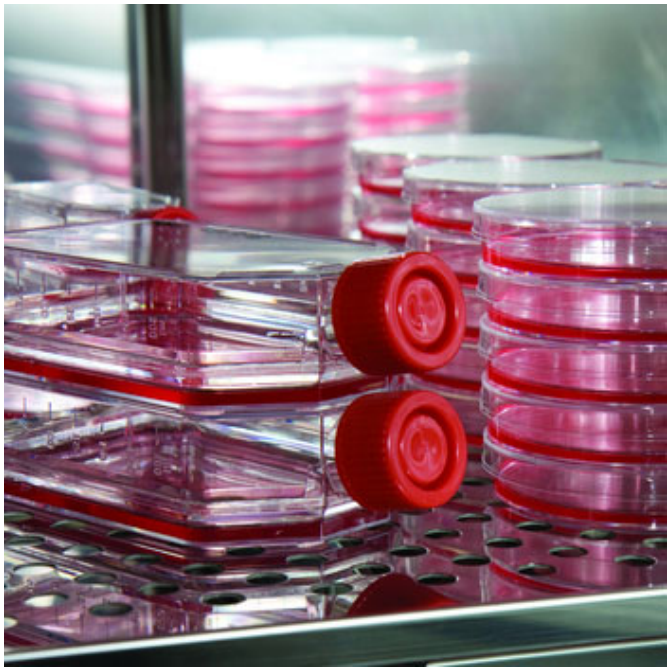
bsi. ISO 13485 Medical Devices Quality Management

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

Outline

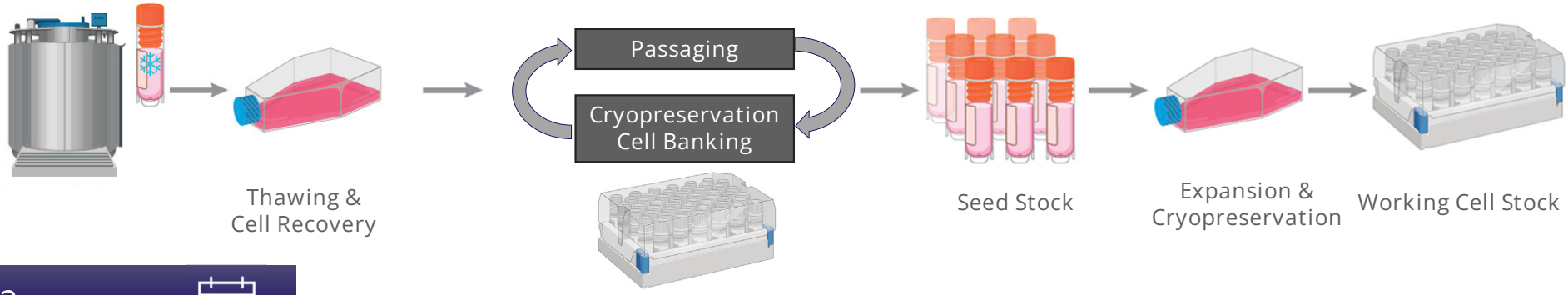
- ThawReady™ introduction
- ThawReady™ solution offerings
- Speaker introduction
- ThawReady™ applications



The Problem

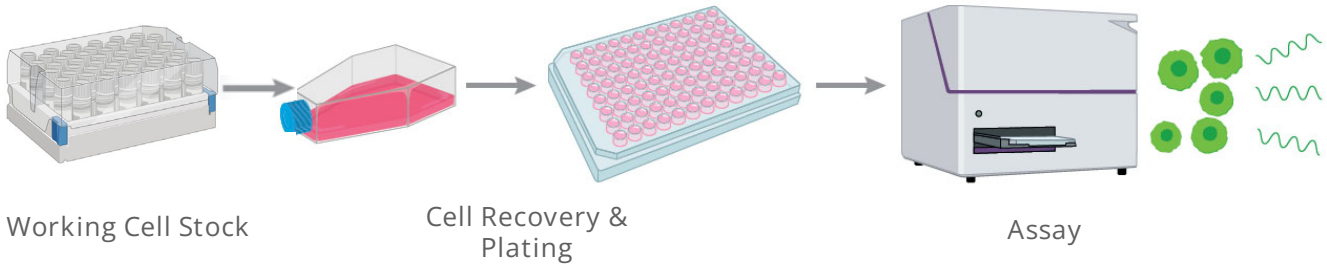
Phase 1 Cell Banking

Months



Phase 2 Assay Workflow

Weeks



Lengthy

Risky

Costly

ThawReady™ Solutions

ATCC® ThawReady™ Assay Ready Cells – From frozen to data in one day

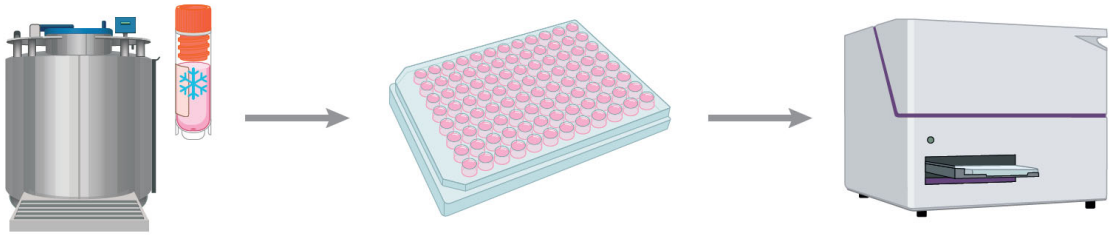


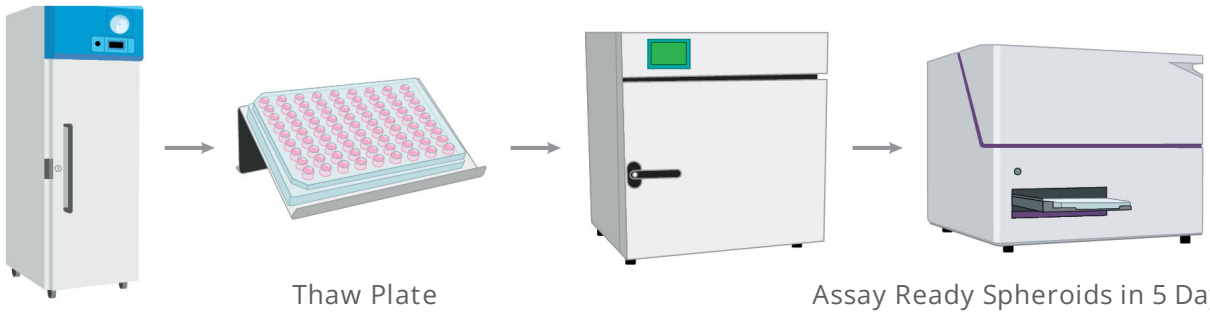
Plate Directly

Assay Ready Immediately

Product Format : Vials; Parental and reporter cell lines

ATCC® No.	Product Designation
TIB-202-NFkB-LUC2-AR™	ThawReady™ THP-1 NF-kB-Luc2
TIB-202-AR™	ThawReady™ THP-1

ATCC® ThawReady™ 3-D Spheroid Kits – A uniform spheroid in every well



Thaw Plate

Assay Ready Spheroids in 5 Days

Product Format : Pre-plated cells; 96-well plates

ATCC® No.	Product Designation
SCM-CCL-185-2PLT™	ThawReady™ A549 Spheroid Kit
SCM-CCL-247-2PLT™	ThawReady™ HCT 116 Spheroid Kit
SCM-HTB-133-2PLT™	ThawReady™ T47D Spheroid Kit

Our Speakers

Fatah Kashanchi, PhD,
Professor, Laboratory of Molecular Virology, George Mason University

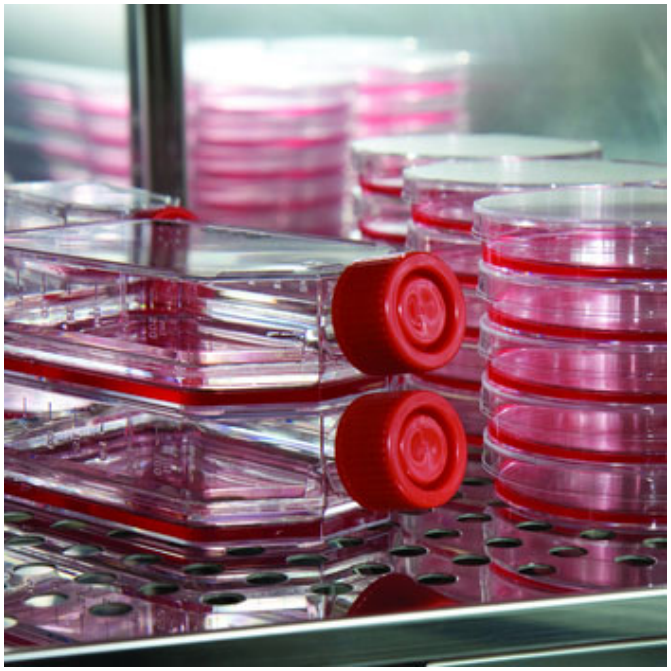
Heather Branscome, PhD,
Senior Scientist, ATCC

Application Data

Heather Branscome, PhD

Overview

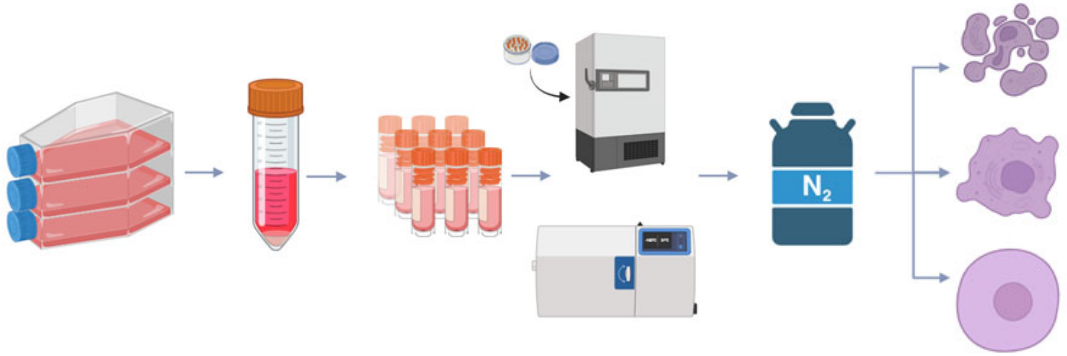
- Challenges with bioassays
- ThawReady™ overview
- Application data
  - Post-thaw recovery
  - Functionality testing using extracellular vesicles (EVs)
  - HIV-1 infection and drugs of abuse



Challenges with Bioassays

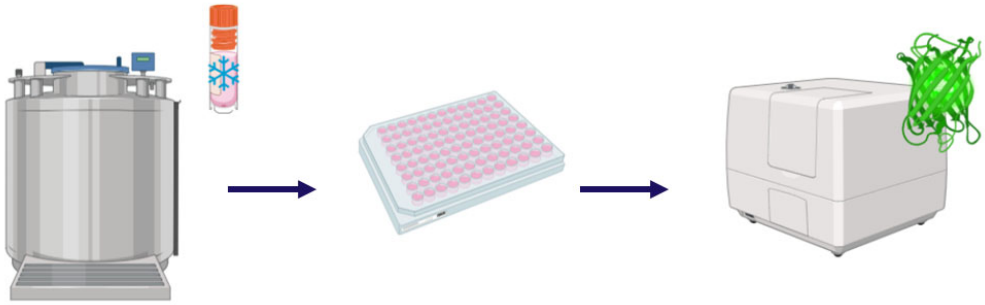
Upstream/downstream challenges

- Time consuming
- Cost and resource dependent
- Introduces variability and genetic drift
- Increased risk of contamination
- Post-thaw recovery period is required



ThawReady™ Solution

- Consumable format
- High reproducibility
- High functionality
- Unique cryobiology



Step 1: Retrieve cells and thaw

Step 2: Plate cells in assay

Step 3: Perform assay

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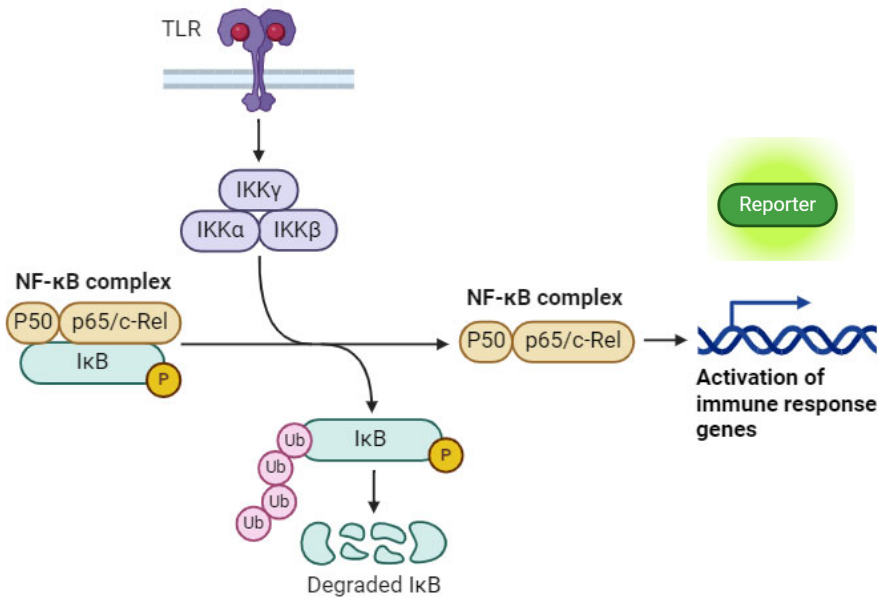
ThawReady™ Overview

THP-1 (ATCC® TIB-202™)

- Monocytic cells derived from the peripheral blood of an acute monocytic leukemia patient
- Versatile and essential for the biomedical research community
- Routinely used in research related to immune system, toxicology/drug development, and cell therapy

ThawReady™ THP-1 NF-kB-Luc2 (ATCC® TIB-202-NFkB-LUC2-AR™)

- Assay-ready, luciferase reporter cell line derived from the THP-1 parental cell line
- Animal by-product free
- Expresses the firefly luciferase gene (luc2) under the control of a NFκB promoter
- Useful for monitoring the activity of NFκB and cellular immune responses in vitro



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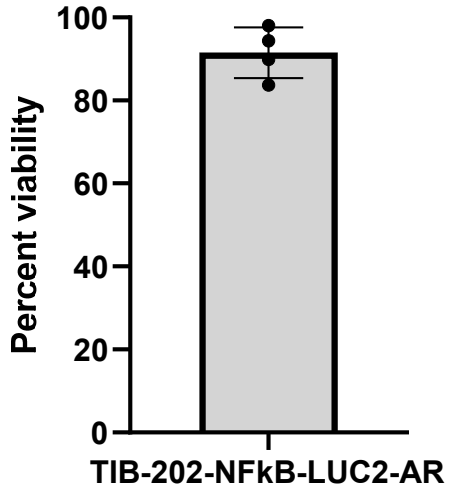
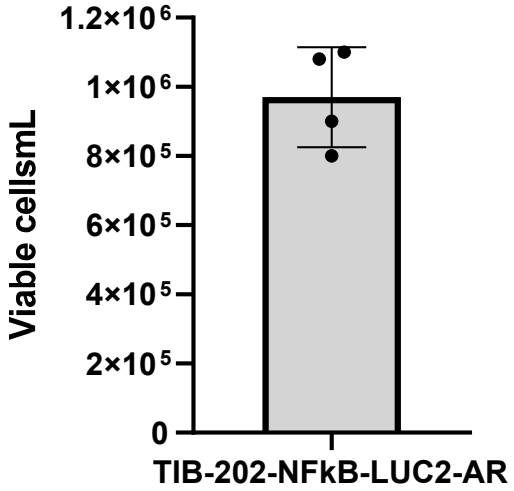
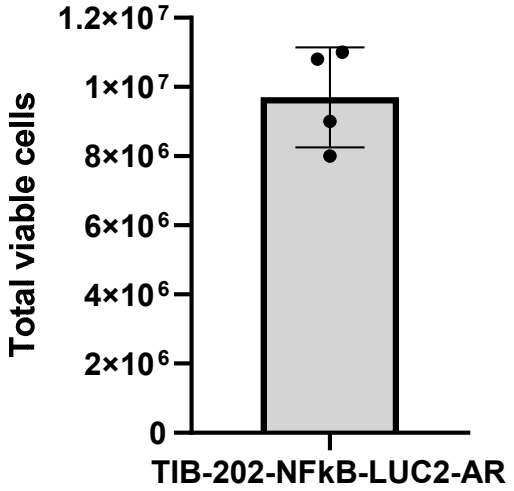
ThawReady™ THP-1 NF-kB-Luc2

ATCC® TIB-202-NFkB-LUC2-AR™

- Reproducible and consistent viability and cell yield
- Measure immediately post-thaw







ThawReady™ THP-1 NF-kB-Luc2

ATCC® TIB-202-NFkB-LUC2-AR™

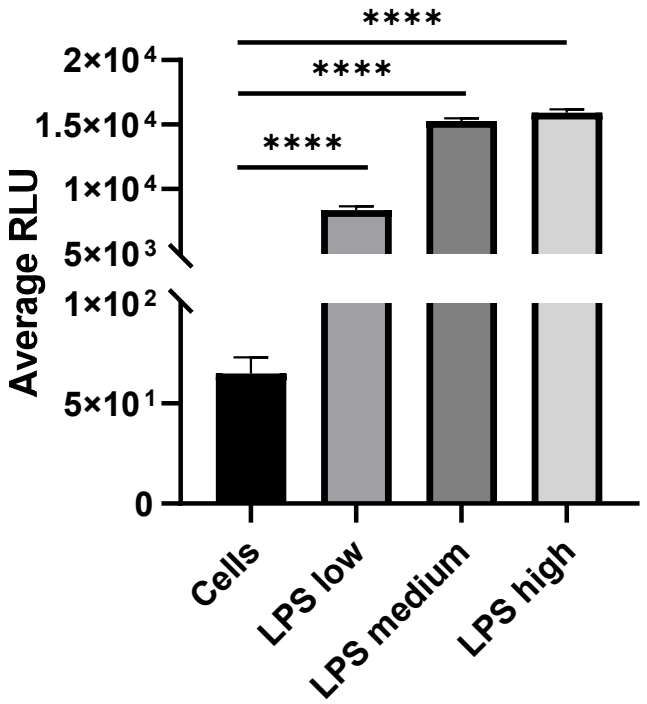
ATCC®

ThawReady™ by ATCC

LPS Challenge Assay

- Average post-thaw viability: 89.9%
- Seeding: Immediate post-thaw
- Seeding density: 50,000 viable cells
- Treatment:
  - LPS (*E. coli* O111:B4)
  - Stimulation period: 3 hours
    - Low dose: 1 µg/mL
    - Medium dose: 10 µg/mL
    - High dose: 25 µg/mL
- Assay/Instrument: Bright-Glo™ assay on BioTek Cytation®

Assayed Directly from Frozen

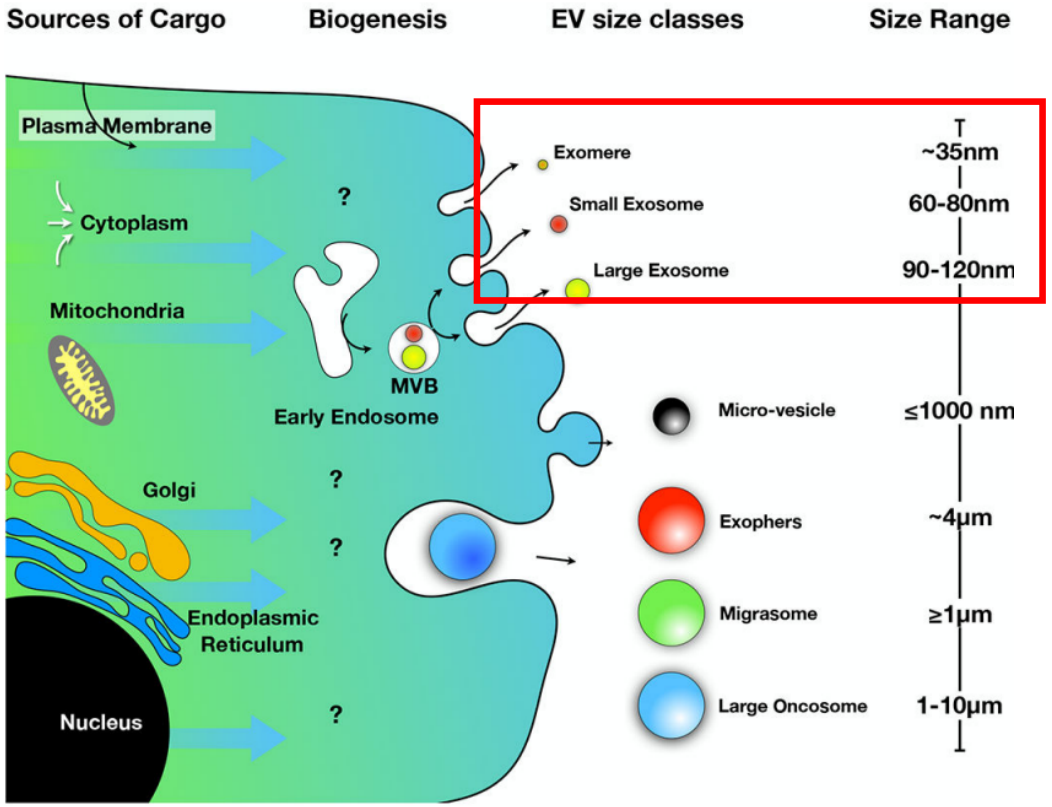


Application Data: Extracellular Vesical (EV) Functionality

Application Data: EV Functionality

Extracellular Vesicles:

- Nano-sized particles (30 nm-1000 nm) released by all cell types
- Carry various components of the cytoplasm and cell membrane
- Mediate intercellular communication (physiological and pathological)
- Can be utilized as diagnostic markers (e.g., cancer EVs) or therapeutic tools (e.g., stem cell EVs)

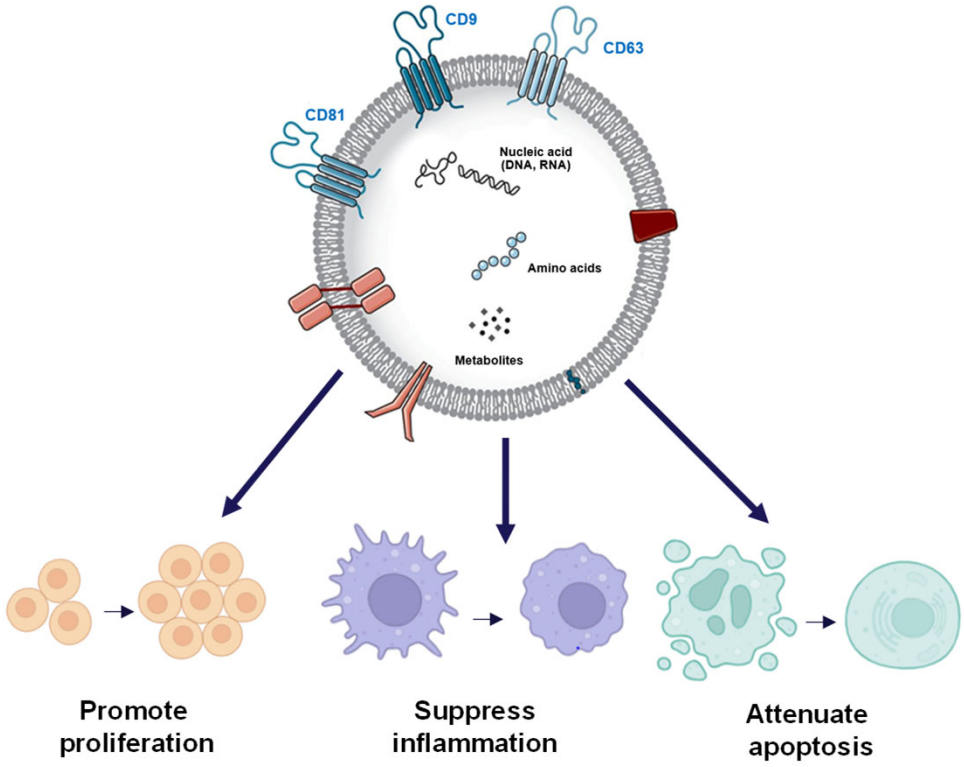


Zijlstra A, Di Vizio D. Nat Cell Biol 20(3): 228-230, 2018. PubMed: 29476154

Application Data: EV Functionality

Stem cell EVs:

- Contribute to tissue repair and regeneration
- Have reduced immunogenicity compared to parental stem cells
- Can be engineered to carry additional therapeutic agents to target damaged cells
- Can be engineered to carry additional therapeutic agents to target cells



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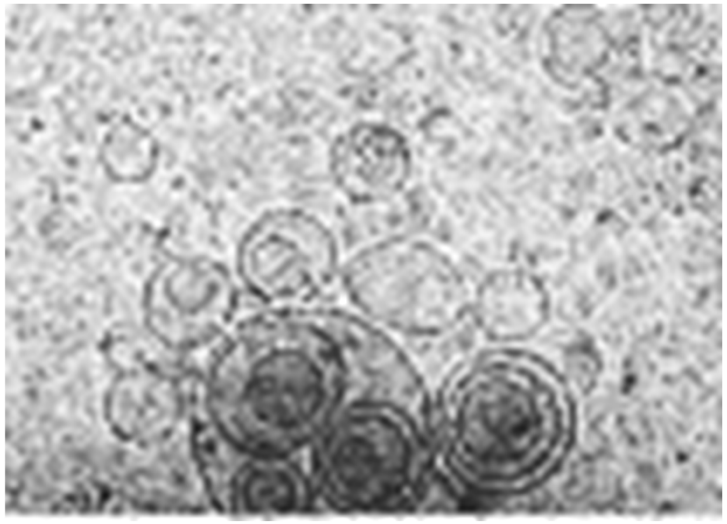
Application Data: EV Functionality

Donor cells:

- Mesenchymal Stem Cells (MSCs) (ATCC® PCS-500-012™)
- Induced Pluripotent Stem Cells (iPSCs) (ATCC® ACS-1019™)
- hTERT-immortalized MSCs (ATCC® SCRC-4000™)

In vitro functionality:

- Pro-migratory
  - Epithelial cells, endothelial cells, fibroblasts
- Pro-angiogenic
  - Mesenchymal stem cells and aortic endothelial cells (co-culture)
- Anti-apoptotic
  - Neurons (ATCC® CRL-2266™)
  - Astrocytes (ATCC® CRL-1718™)
  - Monocyte-derived macrophages (ATCC® TIB-202™)
  - Retinal epithelium (ATCC® CRL-2302™)
- Anti-inflammatory
  - Neurospheres (ATCC® ACS-5003™)



doi.org/10.1007/s11481-019-09865-y

doi.org/10.1038/s41598-022-05848-x

doi.org/10.3390/cells13100861

Application Data: EV Functionality

ATCC® No.	Parental Cell Designation	Model	Applications (pre-clinical)
SCRC-4000-EXM™	hTERT-immortalized adipose-derived mesenchymal stem cell (MSC)	Stem cell (non-cancer)	Therapeutics/ Drug delivery
CRL-1740-EXM™	LNCaP	Carcinoma, prostate	Diagnostics/biomarker discovery
CRL-1435-EXM™	PC-3	Adenocarcinoma, prostate	Diagnostics/biomarker discovery
CCL-185-EXM™	A549	Carcinoma, lung	Diagnostics/biomarker discovery
CCL-247-EXM™	HCT 116	Carcinoma, colorectal	Diagnostics/biomarker discovery

Application Data: ATCC® Stem Cell EVs

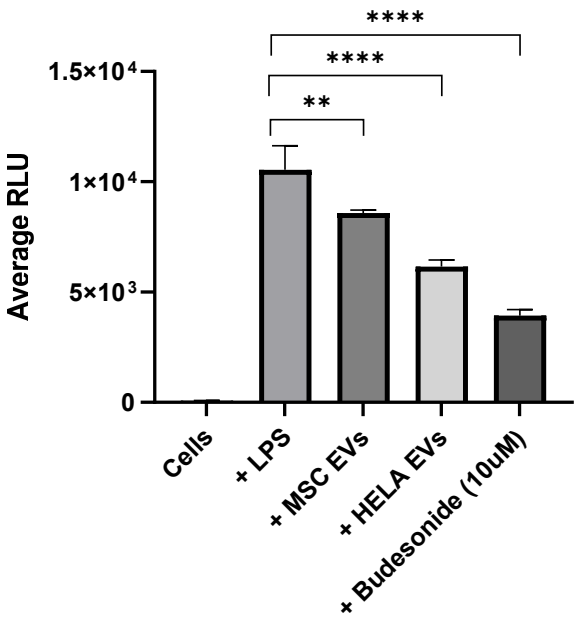
TIB-202-NFkB-LUC2-AR™ EV Function Assay

- Seeding: Immediate post-thaw
- Seeding density: 50,000 viable cells
- Treatment:
  - LPS: *E. coli* O111:B4
  - 3 hour stimulation; 10 µg/mL
  - LPS + stem cell EVs
  - LPS + cancer cell EVs
  - Positive control: Budesonide (10µm)
- Assay/Instrument: Bright-Glo™ assay/CellTiter-Glo® on BioTek Cytation®

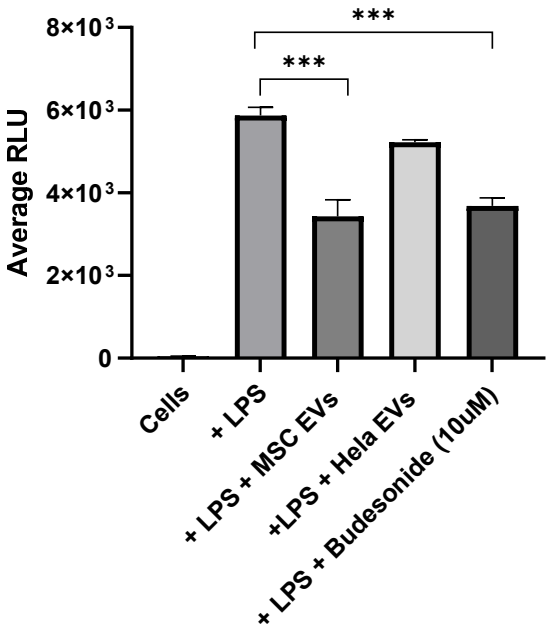
Application Data: ATCC® Stem Cell EVs

ThawReady™ by ATCC

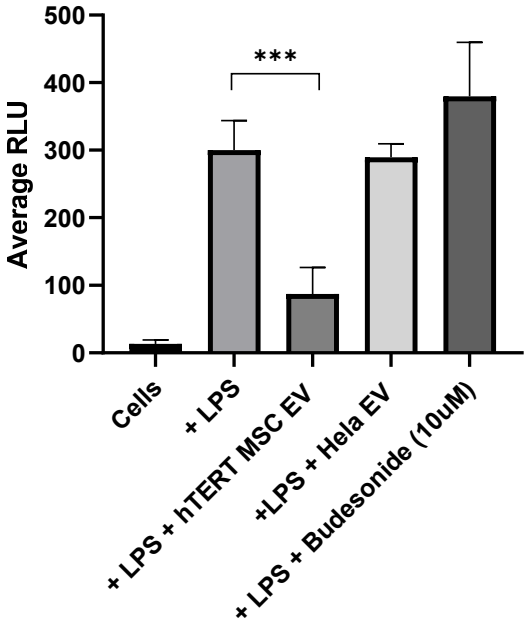
Day 1



Day 2



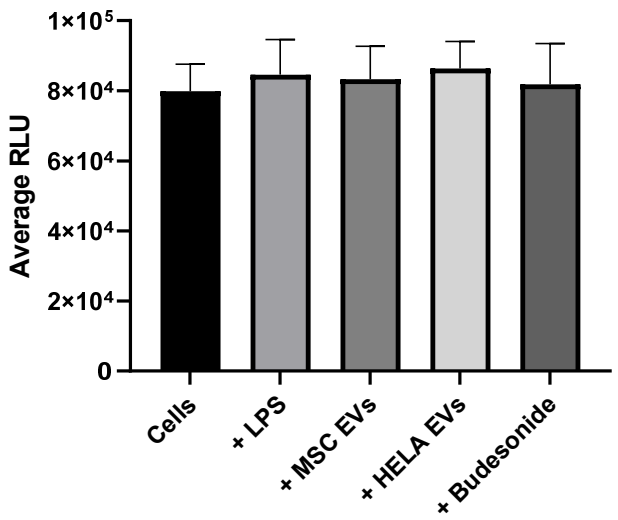
Day 5



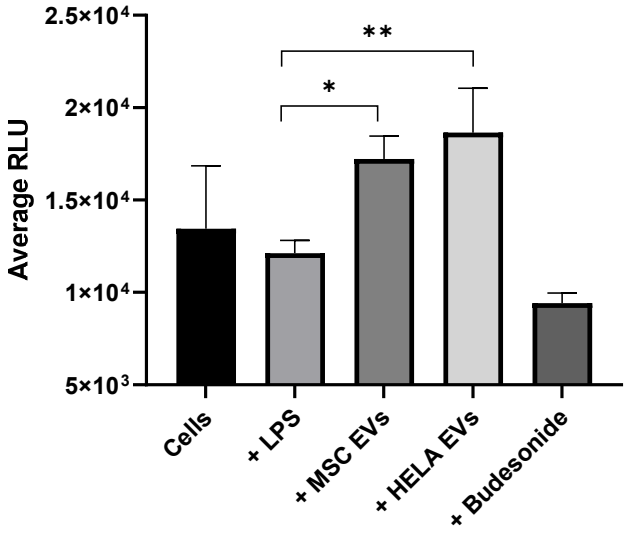
Application Data: ATCC® Stem Cell EVs

ThawReady™ by ATCC

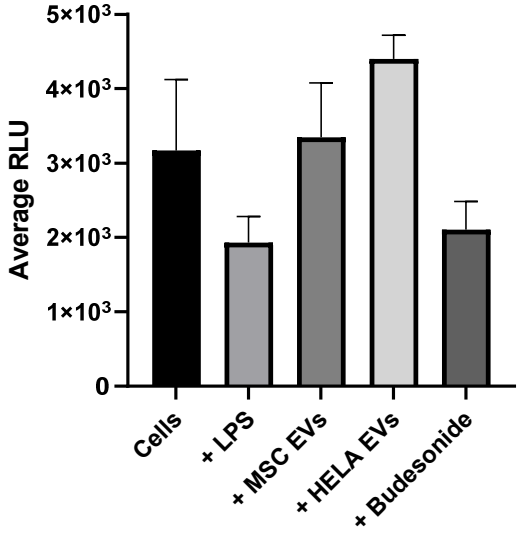
Day 1



Day 4



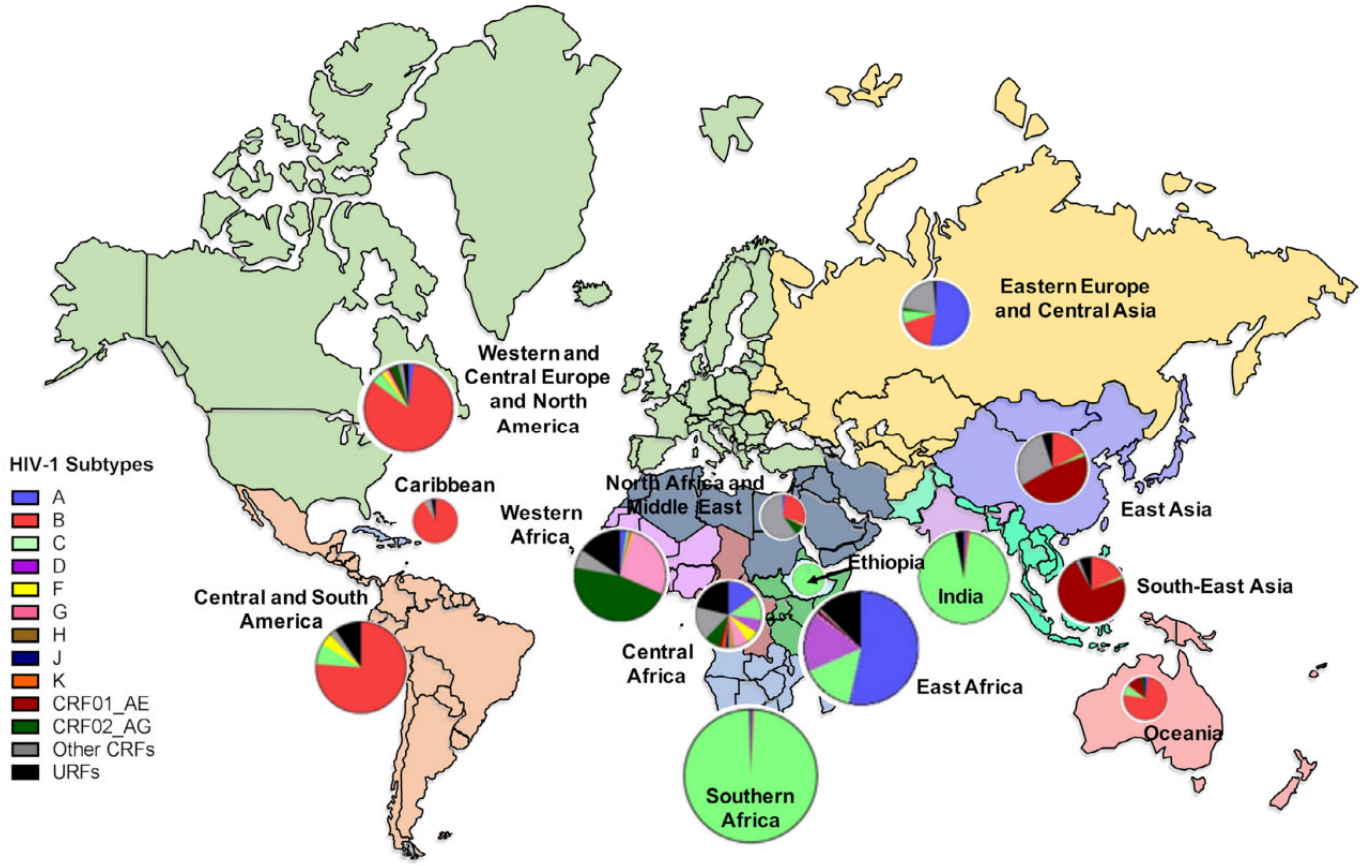
Day 6



GEORGE MASON UNIVERSITY®

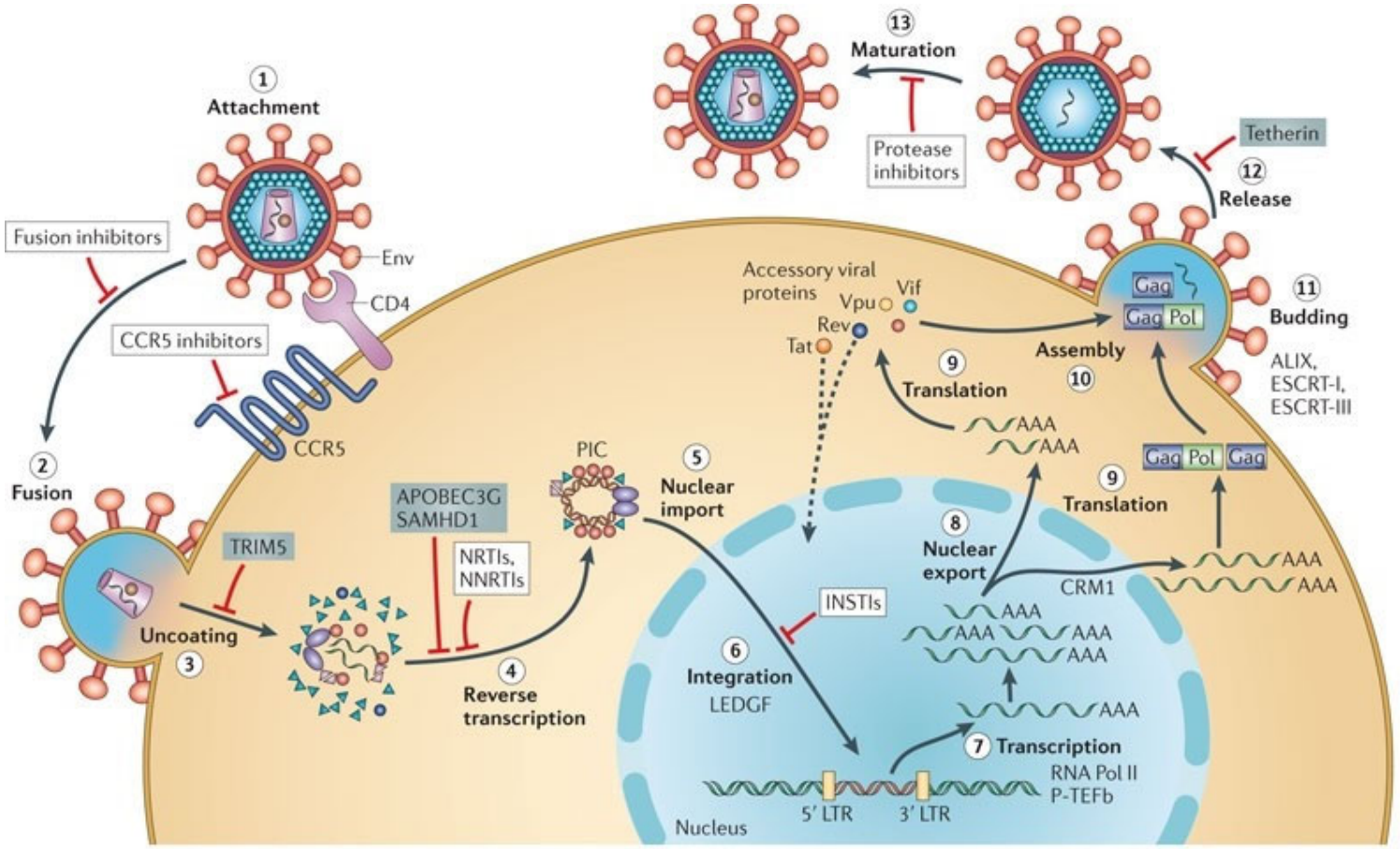
Application Data: HIV-1 Infection

Global prevalence of HIV-1



Gartner MJ, et al. EBioMedicine 53: 102682, 2020. PubMed: 32114391

Application Data: HIV-1 Infection



Engelman A, Cherepanov P. Nature Rev Microbiol 10: 279-290, 2012.

Application Data: HIV-1 Infection

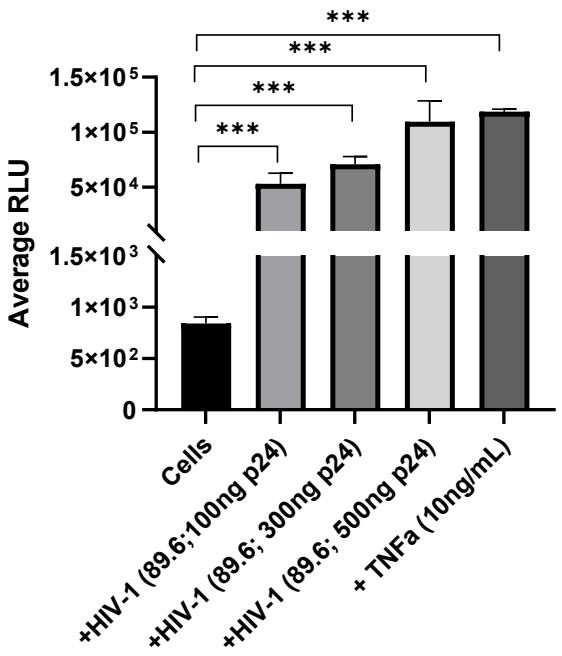
ATCC® TIB-202-NFkB-LUC2-A**R**™ HIV-1 infection assay

- Seeding: Immediate post-thaw
- Seeding density: 50,000 viable cells
- Treatment:
  - PMA (100 ng/mL)
  - HIV-1 dual tropic 89.6 (100, 300, 500 ng p24)
  - cART (5 mM)
  - TNFα (10 ng/mL)
  - CBD (5 uM)
  - HU308 (5 uM)
- Assay/Instrument: Bright-Glo™ assay/GloMax® (multiple reads over 10 minutes)

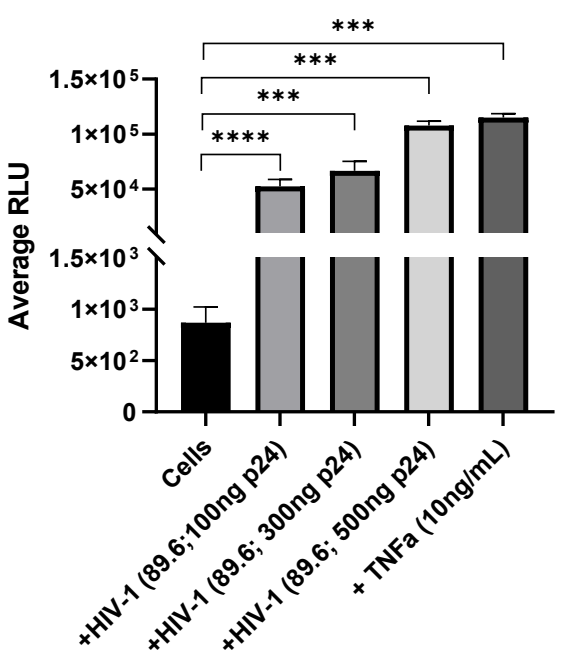
Application Data: HIV-1 Infection

ThawReady™ by ATCC

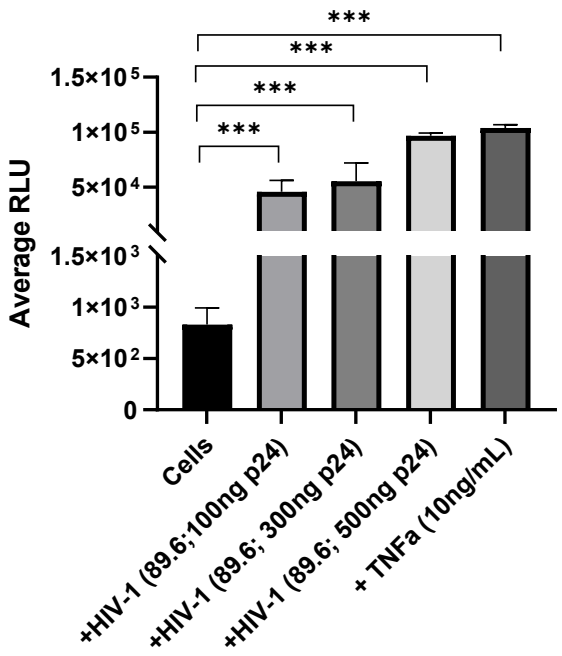
Assay 1



Assay 2



Assay 3



Application Data: HIV-1 Infection

ThawReady™ by ATCC

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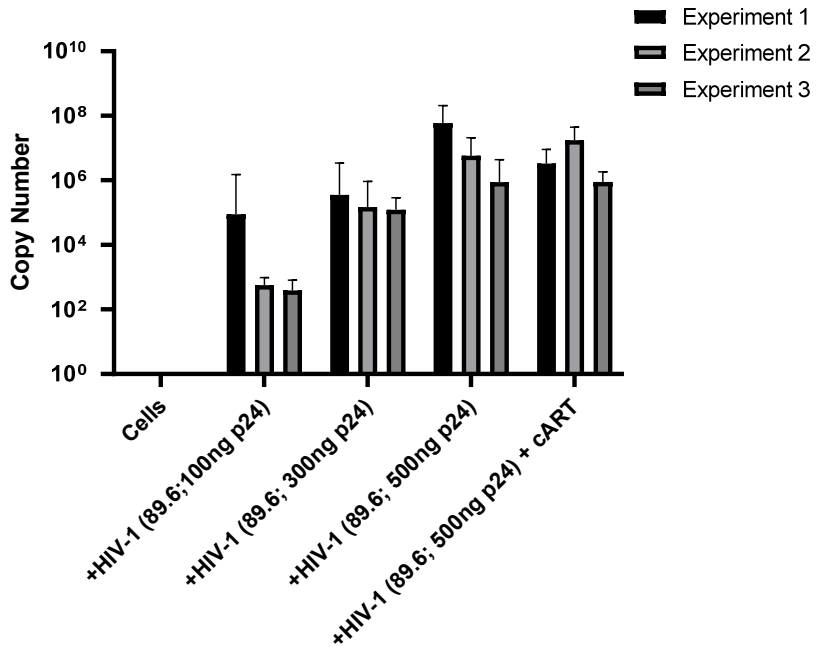

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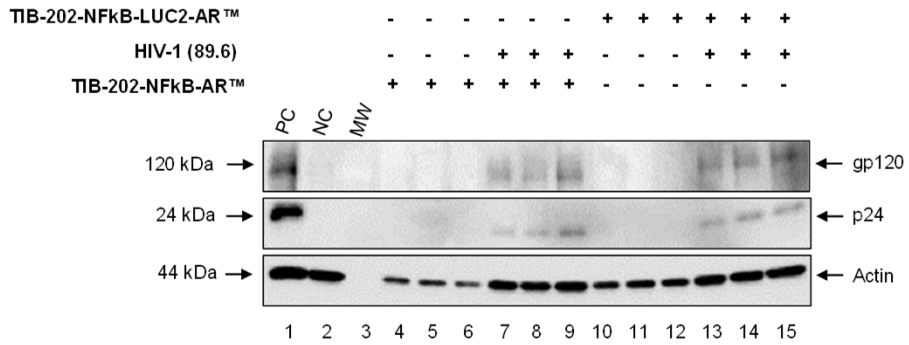

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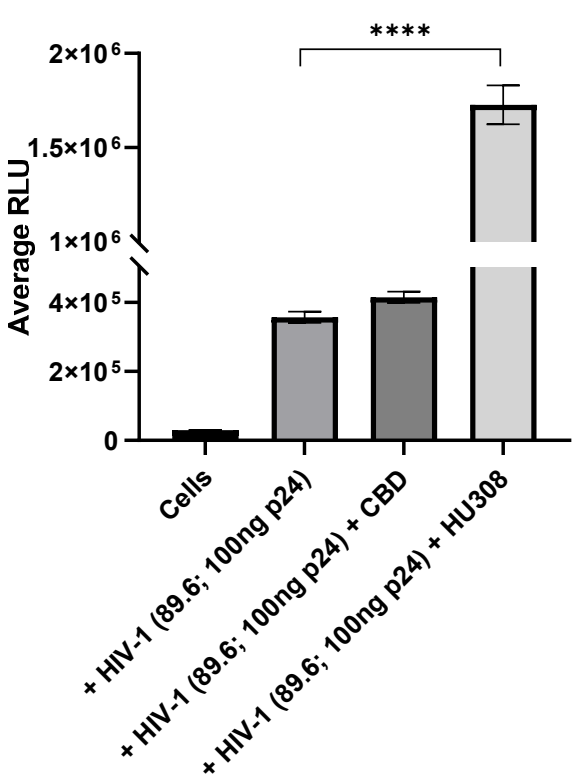
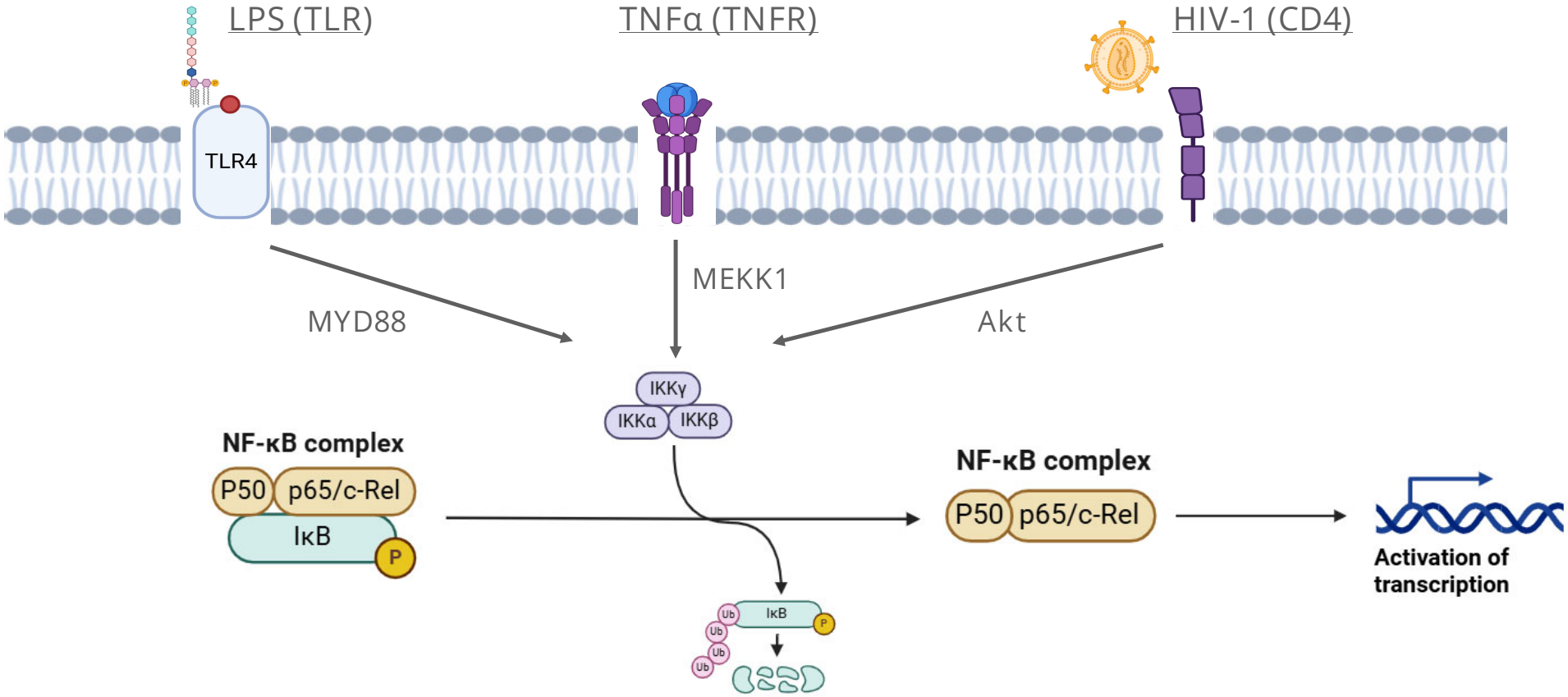


Mechanism

Activation of NFkB signaling via multiple stimulants/receptors



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Summary

- ThawReady™ cells overcome many of the challenges associated with traditional bioassays
- ThawReady™ cells offer high viability, high cell yields, and high functionality in a reproducible manner
- ThawReady™ cells can be used in assays relating to cellular damage (e.g., cancer EVs, viral infection) or cellular repair (e.g., stem cell EVs, drug screening)
- ThawReady™ cells can be used to study various mechanisms of NFκB activation, including TLR4, TNFR, and viral attachment

Acknowledgements

ATCC

- Dr. Joseph Leonelli
- Becky Bradford
- Dr. Heather Couch
- Dr. Sujata Choudhury
- Dr. Fang Tian
- Zach Cuba
- Sarah Swineford
- Jessica Moore
- Dr. Utsav Sharma
- Dr. Brian Shapiro
- Dr. Nilay Chakraborty
- Tiffany Cato

GMU

- Dr. Yuriy Kim
- Kajal Patil
- Anastasia Williams
- Gwen Cox

Learn more about ThawReady™ cells

Visit us online at
www.atcc.org/thawready-cells

Listen to our podcast episode 26:
ThawReady™ – Transforming Cryopreservation for Instant Cell Functionality
www.atcc.org/behindthebiology