Cryopreservation Impact on Functional Recovery of Luciferase Reporter Cells

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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
Luciferase Reporter Cells

Lentiviral transduction

Single cell cloning and expansion

In vitro assays

In vivo modeling
THP-1 Reporter Cells

Let's start with the parent cell line (ATCC® TIB-202™)

Characteristics

- Human monocytic cell line
- Acute monocytic leukemia
- Analogous to peripheral blood mononuclear cells (PBMCs)

Applications

- In vitro cancer modelling
- Monocyte-macrophage differentiation
- Dendritic cell differentiation and modelling

Macrophage Differentiation

## THP-1 Reporter Cells

### ATCC products and advantages

<table>
<thead>
<tr>
<th>Response Element</th>
<th>ATCC No.</th>
<th>Signaling Pathway</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFκB</td>
<td>TIB-202-NFκB-LUC2™</td>
<td>NFκB</td>
<td>Pivotal mediator of inflammatory response</td>
</tr>
<tr>
<td>GAS</td>
<td>TIB-202-GAS-LUC2™</td>
<td>JAK-STAT (Type II)</td>
<td>Initiates immune cell activation and recruitment</td>
</tr>
<tr>
<td>CRE</td>
<td>TIB-202-CRE-LUC2™</td>
<td>cAMP/PKA</td>
<td>Inflammatory mediator and phagocytosis modulator</td>
</tr>
<tr>
<td>ISRE</td>
<td>TIB-202-ISRE-LUC2™</td>
<td>JAK-STAT (Type I)</td>
<td>Initiates immune cell activation and recruitment</td>
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<tr>
<td>AP1</td>
<td>TIB-202-AP1-LUC2™</td>
<td>MAPK/ERK</td>
<td>Regulates innate and adaptive immune response</td>
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<tr>
<td>NFAT</td>
<td>TIB-202-NFAT-LUC2™</td>
<td>Calcineurin-NFAT</td>
<td>Mediates adaptive T and B cell activation</td>
</tr>
</tbody>
</table>

### Key Features

- Fully authenticated parental THP-1 cell line
- High signal-to-noise ratio
- Verified, characterized stable expression
- Easy to culture, robust, and highly sensitive
- Amenable to complex experimentation (combinatorial stimulation, co-culture)
- High density cryopreservation

### Key Benefits

- No concerns about cross-contamination and misidentification, saves time and money
- Clear and more intense results, straightforward data analysis
- Reduced variability, reproducible results
- Ease of use, customer convenience
- Versatile and compatible with multiple platforms
- More viable cells post-thaw
THP-1-NFκB-Luc2 (ATCC® TIB-202-NFκB-LUC2™)

NF-κB signaling

**Complete NF-κB Signaling**

**Simplified NF-κB Signaling**


Prof. David Wallach's Lab, Weizmann Institute of Science, Department of Biomolecular Sciences. https://www.weizmann.ac.il/Biomolecular_Sciences/Wallach/
THP-1-NFkB-Luc2 (ATCC® TIB-202-NFkB-LUC2™)

Cryopreservation of Reporter Cells

Overarching goals:

- Reporter cells must be preserved for long-term storage and transport. Robust recovery and return to normal culture state are essential.

Open questions and potential challenges:

- Are reporter cells impacted by cryopreservation?
- If there is a difference in response, what is the lag time before acceptable responses are possible? Can this be impacted by improved preservation processing?
- Do the modifications needed to create the reporter functionality impact overall cell functionality?
Initial Freeze Experiment

Frozen cells → Thaw processing → Cell plating and 0/24-hr incubation → LPS stimulation → Measure luminescence

Cells in culture → Control processing

0-hr Post-thaw Incubation

24-hr Post-thaw Incubation
LPS-Induced Viability Loss

Frozen cells → Thaw processing → Cell plating and 0/24-hr incubation → LPS stimulation → Measure viability

Cells in culture → Control processing → Cell plating and 0/24-hr incubation

0-hr Post-thaw Incubation

<table>
<thead>
<tr>
<th>Cell Condition</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Unstimulated</td>
<td>100</td>
</tr>
<tr>
<td>Culture Stimulated</td>
<td>80</td>
</tr>
<tr>
<td>Thawed Unstimulated</td>
<td>80</td>
</tr>
<tr>
<td>Thawed Stimulated</td>
<td>60</td>
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</table>

24-hr Post-thaw Incubation

<table>
<thead>
<tr>
<th>Cell Condition</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Unstimulated</td>
<td>80</td>
</tr>
<tr>
<td>Culture Stimulated</td>
<td>60</td>
</tr>
<tr>
<td>Thawed Unstimulated</td>
<td>60</td>
</tr>
<tr>
<td>Thawed Stimulated</td>
<td>40</td>
</tr>
</tbody>
</table>
Changing LPS Dosage and Time Reduces Viability Loss

LPS Stimulation Time

0-hr Post-thaw Incubation
- Thawed (+)
- Culture (+)
- Thawed (-)
- Culture (-)

24-hr Post-thaw Incubation
- Thawed (+)
- Culture (+)
- Thawed (-)
- Culture (-)

LPS Dosage

0-hr Post-thaw Incubation
- Thawed (+)
- Culture (+)
- Thawed (-)
- Culture (-)

24-hr Post-thaw Incubation
- Thawed (+)
- Culture (+)
- Thawed (-)
- Culture (-)
LPS-Induced Cell Death

Video showing the uptake of ethidium bromide by THP-1-NFkB-LUC2 cells during a 60-minute exposure to LPS
Possible biophysical mechanism

**Cryopreservation-induced leaky membrane**
- Membrane phase transitions
- Uptake of impermeable solutes

**Intracellular LPS-induced pyroptosis**
- Faster response than other cell-programmed death responses

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Closing Remarks

- We have identified a response of THP-1-NFκB-LUC2 cells to LPS immediately after thaw that directly impacts assay outcomes.
- THP-1-NFκB-LUC2 reporter cells can produce expected outcomes to LPS exposure within 24 hours post thaw.

Future Work

- Understand the mechanisms causing cell death after LPS exposure to develop targeted cryopreservation strategies to improve post thaw outcomes.
Questions?

Special thanks to Lukas Underwood, who performed the bulk of the work presented.