Illuminate Immuno-Oncology Research with THP-1 Luciferase Reporter Cell Lines

Brian Della Fera, BS
Biologist, ATCC

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 largest, most diverse biological materials and information resource for cell biology – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, advanced cell models
- cGMP biorepository

- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viruses, and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Luciferase-expressing Monocyte Reporter Cell Lines as a Predictive Human Cell-Based Model for In Vitro Immune Activation Studies

Brian Della Fera, Haijun Liu, John Foulke, Fang Tian
ATCC R&D, Gaithersburg, MD 20877, USA

Abstract
Cancer immunotherapy has emerged as an exciting new approach for cancer treatment, and immune-oncology is one of the fastest growing fields in oncology. The development of immunomodulatory drugs and biomarkers calls a clear need for more effective in vitro methods to assess the efficacy of these drugs. Luciferase-expressing reporter cells, such as GALTSEERs, have been used to evaluate immune activation in vitro. However, these assays are limited by the high background noise and the requirement for specialized equipment. To overcome these limitations, we developed a novel reporter cell line, THP-1 Luc2, which stably expresses a luciferase reporter under the control of the IL-8 enhancer/promoter. This cell line allows for rapid, sensitive, and sensitive detection of immune activation in a variety of settings. THP-1 Luc2 cells were able to respond to a wide range of stimuli, including LPS, TNF-α, IFN-γ, and IL-1β, with a robust increase in luciferase activity. This cell line provides a powerful tool for the rapid evaluation of immune activation in vitro, with potential applications in drug discovery and biomarker development.

Background
The immune system plays a critical role in maintaining host defense against pathogens and recognizing and eliminating malignant cells. The ability to accurately and reliably measure immune activation is crucial for the development and optimization of immunotherapies. Current methods for measuring immune activation include flow cytometry and ELISA-based assays, which can be time-consuming and require specialized equipment. Luciferase-expressing reporter cells, such as GALTSEERs, have been used to evaluate immune activation in vitro. However, these assays are limited by the high background noise and the requirement for specialized equipment. To overcome these limitations, we developed a novel reporter cell line, THP-1 Luc2, which stably expresses a luciferase reporter under the control of the IL-8 enhancer/promoter. This cell line allows for rapid, sensitive, and sensitive detection of immune activation in a variety of settings. THP-1 Luc2 cells were able to respond to a wide range of stimuli, including LPS, TNF-α, IFN-γ, and IL-1β, with a robust increase in luciferase activity. This cell line provides a powerful tool for the rapid evaluation of immune activation in vitro, with potential applications in drug discovery and biomarker development.

Results
Characterization of THP-1 Luc2 cells in vitro

Figure 1. Schematic of developing a stable cell line containing the Luc2 gene

Figure 2. Characterization of THP-1 Luc2 cell line

Figure 3. Characterization of luciferase-expressing cell lines

Figure 4. Activation of GAS and NF-κB signaling pathways by various cytokines and TLR ligands in a dose-dependent manner

Figure 5. In vivo detection of IFNγ expression using THP-1 GAS-Luc2 cell line

Figure 6. THP-1 Luc2 cell lines serve as a useful tool to study various arms of the immune response

Conclusion
We have created several human monocytic luciferase reporter cell lines that provide a simple and robust means to measure immune activation through a cell-based measurement. This cell line shows robust performance in deep biological experiments and demonstrates variability in vitro complex experiments. This panel of THP-1 Luc2 cells provides an authentic evaluation tool that can be used in the development of immunomodulatory drugs and biomarkers, studying signaling pathways, and as a safety evaluation tool for new chemicals and drugs.

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Outline: THP-1 Reporter Cell Lines

- Introduction to THP-1 Monocytes and Luciferase Technology
- Development of THP-1 Reporter Cell Lines
- Application of Cell Lines

https://www.hhmi.org/news/hunting-immune-cells-cancer-targets
Introduction Of THP-1 Monocytes And Luciferase Technology
Introduction

Overview

- Background
- THP-1 Project
- Cell Line
- Luciferase
- Response Elements used
Introduction

Product Background

- Immunotherapy has emerged as an exciting new approach for cancer treatment
- Current methods are time consuming, labor intensive, or expensive
- Clear need for a straightforward, human cell-based model that can be implemented as an evaluation tool

https://www.nature.com/collections/gzرنfنqkz
Introduction

Scientific Background

- Applicable in academic research and pharmaceutical R&D
  - Signaling pathways
  - New drug development
  - Safety evaluation tool
- THP-1
  - Established cell line
- Luciferase
  - Well characterized reporter gene system

https://www.novusbio.com/nfkbpathway
Introduction

THP-1 (TIB-202™) Cell Line

- Best surrogate model for studying *in vitro* human monocytes
- Originated from the blood of a leukemia patient
- Differentiate into macrophages
- Homogenous genetic background minimizes variability
Introduction

**Luciferase**

- Derived from fireflies, *Photinus pyralis*
- Higher expression and quicker protein transcription
- Quantified by measuring bioluminescence
- High-throughput, sensitive readings
# Introduction

**Response elements**

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<tr>
<th>Transcription Factor</th>
<th>Signaling Pathway</th>
<th>Function</th>
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<td>AP-1</td>
<td>MAPK/ERK</td>
<td>Regulates innate and adaptive immune response</td>
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<tr>
<td>CRE</td>
<td>cAMP/PKA</td>
<td>Inflammatory mediator and phagocytosis modulator</td>
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<td>JAK-STAT (Type II)</td>
<td>Initiates immune cell activation and recruitment</td>
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<td>Calcineurin-NFAT</td>
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<td>NF-κB</td>
<td>NF-κB</td>
<td>Pivotal mediator of inflammatory response</td>
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Development of THP-1 Reporter Cell Lines
Development

- Workflow
- Authentication
- Verification testing
Workflow for Developing Cell Lines

1. **Viral transduction**
   - Lenti-Luc2 plasmids
   - Viral Transfection
   - Collect viral particle
   - Virus titration

2. **Recovery post-selection**
   - Infect parental cells
   - Evaluate the transduced cell pool
   - Cell sorting
   - Expansion

3. **Single clone isolation**
   - Antibiotic selection
   - Screen luc activity/clone selection
   - Cell sorting
   - Expansion

4. **Screen luc activity/clone selection**
   - Screen and validate clones

5. **Clonal expansion/functional testing**
   - Growth Kinetics
   - Single Clone Populations

Diagram:
- Viral Transfection
- Antibiotic selection
- Cell sorting
- Expansion
- Screen and validate clones
### Authentication – STR Profiling

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<th>THP-1 PARENTAL</th>
<th>THP-1 GAS-LUC2</th>
<th>THP-1 NFκB-LUC2</th>
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<th>THP-1 CRE-LUC2</th>
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No change in STR markers
Authentication – Morphology

THP-1 Parental

THP-1 AP-1 Luc2

THP-1 ISRE Luc2

THP-1 CRE Luc2

THP-1 NFAT Luc2
Authentication – Contaminants and CO1 Barcode

- Mycoplasma
  - Negative

- Bacterial Contamination
  - Negative

- CO1 Barcode
  - Human
  - No cross contamination
Verification Testing

- Induction curve validates the linear correlation between bioluminescence and cell number
- Weekly stability demonstrates the consistent expression of luciferase

![Graph showing THP-1 GAS-Luc2 Induction Curve]

- $R^2 = 0.9706$

![Graph showing THP-1 CRE-Luc2 Weekly Stability]

- Fold Change
Application Data
Application Data

- Exogenous Stimulation
- Small Molecule Inhibitors
- T Cell Proliferation Analysis
Exogenous Stimulation

- **THP-1 GAS-Luc2 IFNα Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3

- **THP-1 GAS-Luc2 IFNβ Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3

- **THP-1 GAS-Luc2 IFNγ Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3

- **THP-1 ISRE-Luc2 IFNα Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3

- **THP-1 ISRE-Luc2 IFNβ Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3

- **THP-1 ISRE-Luc2 IFNγ Stimulation**
  - Fold Change vs. Concentration (ng/mL)
  - n=3
Exogenous Stimulation

THP-1 NFκB-Luc2
LPS Stimulation

THP-1 NFκB-Luc2
TNFα Stimulation

THP-1 NFκB-LUC2
HMGB1 Stimulation

THP-1 AP1-LUC2
PMA Stimulation

THP-1 AP1-Luc2
LPS Stimulation

n=3 n=3 n=3 n=3
Exogenous Stimulation

THP-1 CRE-LUC2
Foreskolin Stimulation

THP-1 NFAT-Luc2
PMA + Ionomycin Stimulation

THP-1 CRE-LUC2
PMA + Ionomycin Stimulation

THP-1 NFAT-Luc2
ConA + Ionomycin Stimulation

Exponential Stimulation

THP-1 CRE-LUC2
Foreskolin Stimulation

THP-1 NFAT-Luc2
PMA + Ionomycin Stimulation

THP-1 CRE-LUC2
PMA + Ionomycin Stimulation

THP-1 NFAT-Luc2
ConA + Ionomycin Stimulation
Small Molecule Inhibitor Effects on Expression

THP-1 ISRE-Luc2
CYT387 Inhibitory Study

THP-1 ISRE-Luc2
CYT387 Cytotoxicity

Fold Change

% Viability

Concentration CYT387 (μg/mL)

Concentration CYT387 (μg/mL)
Small Molecule Inhibitor Effects on Expression

**THP-1 GAS-Luc2 CYT387 Inhibitory Study**

- **Fold Change**
  - Concentration CYT387 (µg/mL): 0, 0.01, 0.1, 1, 10
  - n=3

**THP-1 GAS-Luc2 CYT387 Cytotoxicity**

- **% Viability**
  - Concentration CYT387 (µg/mL): 0, 0.01, 0.1, 1, 10
  - n=3
Small Molecule Inhibitor Effects on Expression

**THP-1 NFkB-Luc2 Bay11-7082 Inhibitory Study**

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n=3

**THP-1 NFkB-Luc2 Bay11-7082 Cytotoxicity**

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<th>Concentration (μg/mL)</th>
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<td>5</td>
<td>98 ± 4</td>
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<td>95 ± 3</td>
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n=3
PBMC T Cell Proliferation Study

Using stimulated T cell supernatant to study IFN expression with THP-1 GAS-Luc2
CD8+ T Cell Proliferation Protocol

- **Stimulation Reagents**
  - MACS™ – Miltenyi Biotec© – Antibody based reagent that mimics a superantigen binding to TCR
  - Anti-CD3 coated well
  - Anti-CD3/CD28 coated well

- **Supernatant Removed**
  - IFNγ concentration quantified by immunoassay
  - Cultured with THP-1 GAS-Luc2 to measure expression
CD8+ T Cell Cytokine Expression Quantification

**THP-1 GAS-Luc2 Luciferase Expression**

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**IFNγ Expression**

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Conclusion
Available Cell Lines

- All 6 cell lines are available for purchase at www.ATCC.org

<table>
<thead>
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<th>Designation</th>
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<td>THP-1 NF-κB-Luc2</td>
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Conclusion

Summary

- THP-1 reporter cell line will save you time and money
  - No need to undergo the development process
  - Performance already tested

- Completed verification and QC testing
  - Tested activation against appropriate stimuli
  - Cells are well-authenticated and contaminant free

- The reporter cell lines give the scientific community a straightforward, robust evaluation tool
  - Signaling pathway identification
  - Immunomodulatory drug screening
  - Safety assessment

www.atcc.org/advancedimmunology