Description

NLRP3 Knockdown THP-1 Cell Pool is a cell pool of THP-1 human monocytic cells. This cell pool was generated by using NLRP3 Human shRNA Lentivirus (BPS Bioscience #82122) to create a THP-1 cell pool with stable knockdown of NLRP3 (NLR family pyrin domain containing 3). THP-1 cells are the most commonly used model cell line in the study of inflammasome activation as they express high levels of NLRP3 and pro-caspase-1.

Background

NOD, LRR and pyrin domain containing 3 (NLRP3), also known as NALP3 and cryopyrin, is a pattern recognition receptor (PRR) of the NRL (NOD-like receptor) subfamily. It is involved in detection of microbes, endogenous and exogenous stress signals. It is expressed in macrophages and when bound to PYCARD (adaptor ASC protein) forms a caspase-1 activating complex named NRLP3 inflammasome. NLRP3 detects uric acid and extracellular ATP in damaged, and once activated it leads to an immune response. Upon activation NLRP3 inflammasome releases its inactive form partners, HSP90 and SGT1, and binds to PYCARD and caspase-1. Caspase-1 initiates the processing and release of pro-inflammatory cytokines IL-1β and IL-18 and gasdermin D-mediated pyroptotic cell death. Mutations in NLRP3 are known to cause autoinflammatory and neuroinflammatory diseases, such as Alzheimer's, Parkinson's, and prion disease. NLRP3 is the most extensively studied inflammasome protein to date due to its array of activators and aberrant activation in several inflammatory diseases. Studies into its function and inhibition can lead to the development of therapeutic avenues for the treatment of auto-inflammatory diseases.

Application

Study the effect of NLRP3 knockdown on cellular function.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

THP-1, human monocyte, suspension

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2M	BPS Bioscience #78181

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Stability

As this is a cell pool and not a cell line, BPS Bioscience cannot guarantee the stability of the genetic modifications over time. Clonal selection can be performed. We recommend freezing cell vials very early on and growing the cells for a limited number of passages. Cells should be cultured using Growth Media, which contain selection antibiotics.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is highly recommended. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do not contain selective antibiotics. However, Growth Media do contain selective antibiotics, which are used for maintaining selective pressure on the cell population expressing the gene of interest over passages. Cells should be grown at 37°C with 5% CO_2.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2M (BPS Bioscience #7818):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of puromycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2**,** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml, in Growth Medium 2M.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 2 x 10^6 cells/ml, in Growth Medium 2M. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

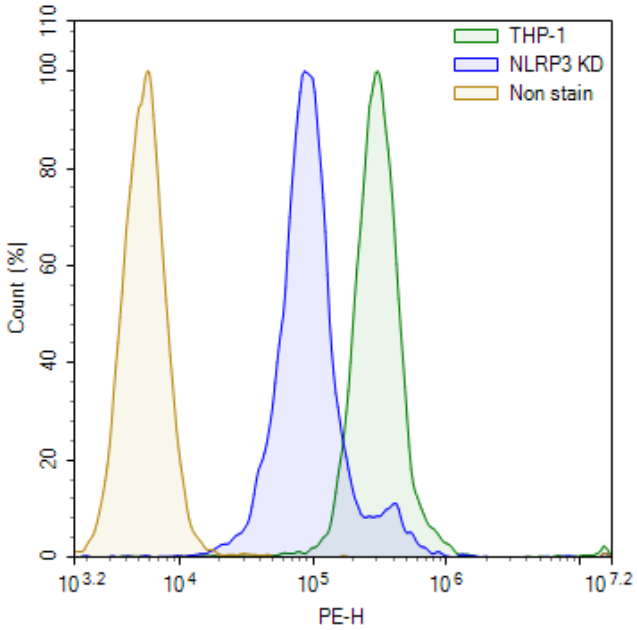

Figure 1. NLRP3 expression levels in NLRP3 Knockdown THP-1 Cell Pool.
Parental THP-1 cells (green) and NLRP3 Knockdown THP- 1 cells (blue) were stained with an anti-human NLRP3 polyclonal antibody (Invitrogen #PA5-79740) and analyzed by flow cytometry. Non-stained cells were used as control (yellow). The Y-axis represents the % cell number. The X-axis indicates the intensity of PE signal.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References
Swanson K., *et al.*, 2019 *Nature Reviews Immunology* 19:477-489.

Related Products

Products	*Catalog #*	*Size*
NLRP3 CRISPR/Cas9 Lentivirus (Integrating)	78545	500 µl x 2
NLRP3 CRISPR/Cas9 Lentivirus (Non-Integrating)	78546	500 µl x 2
NLRP3 Human shRNA Lentivirus	82122	500 µl x 2
NLRP3 (NALP3), His-FLAG-Tags (Sf9-derived) Recombinant	40741	10 µg