Description

The Membrane-Bound TNFα CHO Cell Line is a clonal CHO cell line stably expressing human membrane-bound TNFα (tumor necrosis factor alpha) driven by an EF1a promoter. The cells were generated by transduction with Membrane-Bound TNFα (mTNFα) Lentivirus (#78955).

Background

Tumor necrosis factor (TNF, also known as TNFα) is produced predominantly by activated macrophages and T lymphocytes. It has been identified as a key regulator in inflammatory and immune responses. TNF signaling pathways are triggered by binding to one of two distinct receptors, designated TNFR1 (TNF receptor 1) and TNFR2, which are differentially regulated on various cell types in normal and diseased tissues. TNFα exists in both a trimeric membrane-bound form (mTNFα) and as a soluble protein. TNFα is synthetized in a precursor form, a cell surface type II transmembrane protein, which is cleaved by metalloproteinases such as TACE (TNFα converting enzyme) into a soluble peptide. Soluble TNFα can then bind to its receptors and activate downstream signaling pathways. Transmembrane TNFα can also bind to TNFα receptors and induce cellular responses. For instance, it enhances cytotoxicity of NK cells, while in the liver it can trigger hepatitis. Anti-TNFα antibodies can bind to the mTNFα and trigger antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) to destroy the mTNFα-expressing inflammatory cells, being a promising therapy for inflammatory diseases.

Application(s)

- Use as target cells to measure anti-TNFα antibodies ADCC (antibody dependent cellular cytotoxicity) responses.
- Measuring antibody binding affinity to mTNFα.

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

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

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 3	BPS Bioscience #60186
Growth Medium 3J	BPS Bioscience #79974

Materials Used in Cellular Assay

Name	Ordering Information
Growth Medium 2A	BPS Bioscience #60190
Assay Medium 2A	BPS Bioscience #79621
Thaw Medium 2	BPS Bioscience #60184
ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60541
Infliximab Recombinant Monoclonal Antibody	ThermoFisher #MA5-41776
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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.

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 to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3J (BPS Bioscience #79974):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 5 µg/ml of Puromycin.

Media required for Cellular Assay:

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

Growth Medium 2A (BPS Bioscience #60190):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Assay Medium 2A (BPS Bioscience #79621):
RPMI 1640 medium supplemented with 10% low IgG FBS, 1% Penicillin/Streptomycin.

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 3.

   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 3.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 48-72 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 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 100% confluency. Switch to Growth Medium 3J for passage.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA, following the volumes recommended for the cell vessel being used.

2. Once the cells have detached, add Growth Medium 3J and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3J.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 once or twice a week.

Cell Freezing

1. After detachment, spin down the cells at 300 *x g* for 5 minutes.

2. Remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

3. 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.

4. 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.

Validation Data

A. Cell surface expression of TNFα

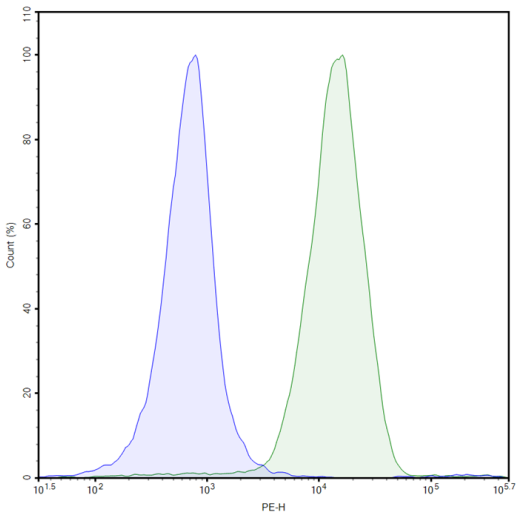

Figure 1: Cell surface expression of TNFα in Membrane-Bound TNFα CHO Cell Line assessed by flow cytometry.
Membrane-Bound TNFα CHO cells (green) and parental CHO-K1 (blue) were stained with Infliximab Recombinant Monoclonal Antibody (ThermoFisher #MA5-41776) followed by Anti-Human IgG Fc, PE (clone: HP6017) (Cedarlane #CL6017PE) and analyzed by flow cytometry. The y axis represents the cell %, while the x axis indicates PE intensity.

B. ADCC activity of ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat Cell Line co-cultured with Membrane-Bound TNFα CHO Cell Line in response to an anti-TNFα antibody.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Antibody Treated Cells”, “Non-Specific Antibody Treated Cells”, “Luminescence Background” and “Untreated Cells” conditions.
- This assay requires ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells, which should be ready 48 hours prior to the assay.

1. Thaw and grow ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells in Thaw Medium 2 and Growth Medium 2A, respectively (for detailed information please refer to the datasheet of this cell line, ADCC Cell Line - High Affinity (bpsbioscience.com)).

Day 1:

1. Switch culture medium of ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells to Assay Medium 2A for 24-48 hours.

Day 2 or 3:

1. Prepare a cell suspension of Membrane-Bound TNFα CHO cells at 1.0 x 10^5 cells/ml in Growth Medium 3J (100 µl/well).

2. Plate 100 µl of Membrane-Bound TNFα CHO cell suspension into each well of a white clear-bottom 96-well tissue culture plate. Leave a few wells empty (no cells) for the "Background Luminescence" control.

3. Incubate at 37°C in a CO_2 incubator for 12-24 hours.

Day 3 or 4:

1. Prepare the test antibody and the non-specific control antibody at 2x the desired final concentrations in Assay Medium 2A (50 µl/well).

2. Remove the medium from the wells of Membrane-Bound TNFα CHO cells.

3. Add 50 µl of diluted test antibodies to "Antibody Treated Cells" wells.

4. Add 50 µl of diluted non-specific control antibody to the "Non-specific Antibody Treated Cells" wells.

5. Add 50 µl of Assay Medium 2A to the "Untreated Cells" and "Luminescence Background" controls.

6. Incubate the plate at 37°C in a CO_2 incubator for 30 minutes to 1 hour.

7. Collect and resuspend ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells at 5 x 10^5 cells/ml in Assay Medium 2A (50 µl/well).

8. Add 50 µl of diluted ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells to the "Antibody Treated Cells", Non-specific Antibody Treated Cells" and "Untreated Cells" wells.

9. Add 50 µl of Assay Medium 2A to the "Luminescence Background" control wells (for determining background luminescence).

10. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

11. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

12. Incubate with gentle agitation at RT for ~15 to 30 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NFAT luciferase reporter expression is the background-subtracted luminescence of "Antibody Treated Cells" well divided by the average background-subtracted luminescence of "Untreated Cells" control wells.

$$Fold\ Induction = \frac{Lum(Antibody\ treated - Background)}{Lum(Untreated - Background)}$$

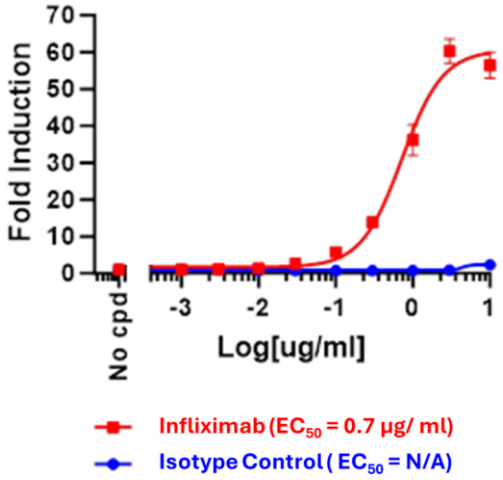

Figure 2: ADCC response of Membrane-Bound TNFα CHO Cell Line co-cultured with ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat Cell Line in the presence of Infliximab Recombinant Monoclonal Antibody, an anti-TNFα antibody.
ADCC Bioassay Effector Cell V Variant (High Affinity)/NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #60541) were incubated with Infliximab Recombinant Monoclonal Antibody (ThermoFisher #MA5-41776) or an isotype control antibody and co-cultured with Membrane-Bound TNFα CHO target cells. NFAT-responsive luciferase activity was measured with ONE-Step™ Luciferase Assay System.

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

References

Wang F., *et al.*, 2017 *Mol Med Rep* 16: 1021-1030.
Horiuchi T., *et al.*, 2010 *Rheumatology* 49(7):1215-1228.

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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.

Related Products

Products	*Catalog #*	*Size*
Membrane-Bound TNFα (mTNFα) Lentivirus	78955	500 µl x 2
Human Tumor Necrosis Factor-alpha Recombinant	90244	10 µg/50 µg
TNFR2 HEK293 Cell Line	78828	2 vials
TNFR2 Lentivirus	78765	500 µl x 2
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag Recombinant	79363	100 µg
TNFR2:TNFalpha[Biotinylated] Inhibitor Screening Assay Kit	79756	96 reactions

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