Description

The Membrane APRIL CHO Cell Line is a clonal CHO cell line stably expressing an engineered, cleavage-resistant form of APRIL (A proliferation-inducing ligand), that enforces its cell surface localization.

This cell line has been validated by flow cytometry, and in a co-culture assay with BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line (#79755) in the presence of Sibeprenlimab.

Background

APRIL (A proliferation-inducing ligand), also known as TNSF13 or CD256, activates a signaling pathway that participates in the regulation of B-cell development, autoimmunity, and long-term plasma cell survival. This protein is a member of the tumor necrosis factor (TNF) ligand family and a ligand for BCMA/ TNFRSF17. APRIL expression is significantly elevated in multiple myeloma (MM), and it has been demonstrated that activation of BCMA by APRIL promotes tumor growth, chemoresistance, and immunosuppression in the bone marrow microenvironment. Anti-APRIL monoclonal antibodies, such as sibeprenlimab and zigakibart, have recently progressed to clinical trials for Immunoglobulin A Nephropathy (IgAN) and MM, respectively. Macrophages express a membrane-bound form of APRIL, which may be involved in immunomodulation. The study of membrane-bound APRIL may be of relevance for the treatment of inflammatory disorders.

Application(s)

- Measure antibody binding to the membrane form of APRIL.
- Use in co-culture experiments to assay APRIL-directed biologics.

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 1A	BPS Bioscience #79528
Thaw Medium 1	BPS Bioscience #60187
BAFF/April Responsive BCMA-NFkB Luciferase Reporter HEK293 Cell Line	BPS Bioscience #79755
Anti-APRIL Neutralizing Antibody (Sibeprenlimab)	BPS Bioscience #102736
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 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1A (BPS Bioscience #79528):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B and 400 µg/ml G418.

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% confluence. 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 April

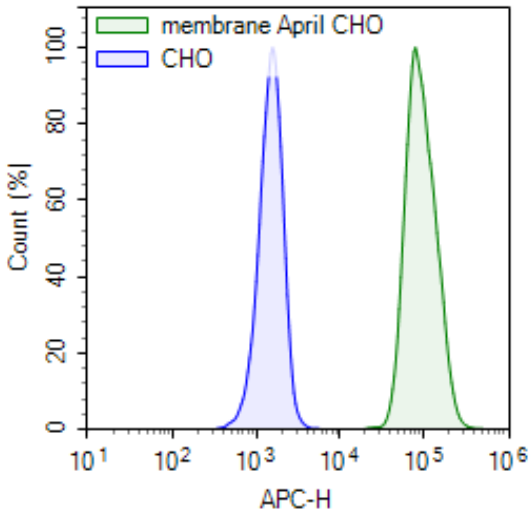

Figure 1: Cell surface expression of APRIL in Membrane APRIL CHO Cell Line assessed by flow cytometry.
Membrane APRIL CHO cells (green) and parental CHO-K1 (blue) were stained with Sibeprenlimab followed by Alexa Fluor® 647 anti-human IgG Fc Recombinant Antibody (BioLegend #409320) and analyzed by flow cytometry. The y axis represents the cell %, while the x axis indicates Alexa Fluor 647 intensity.

B. Inhibition of membrane APRIL-induced NF-κB activity by anti-APRIL antibody Sibeprenlimab, in the BAFF/APRIL Responsive BCMA/NF-κB Reporter HEK293 Cell Line.

- 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.
- We recommend using a non-specific antibody as control.
- The assay should include “Antibody Treated Cells”, “Luminescence Background” and “Untreated Cells” conditions.
- This assay requires BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line (#79755).

1. Thaw and grow BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells in Thaw Medium 1 and Growth Medium 1A, respectively (for detailed information please refer to the datasheet of this cell line).

Day 1:

1. Prepare a cell suspension of BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells at 3.0 x 10^5 cells/ml in Thaw Medium 1 (100 µl/well).

2. Plate 100 µl of BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cell suspension into each well of a white clear-bottom 96-well tissue culture plate. Leave a few wells empty (no cells) for the “Luminescence Background” control.

Day 2:

1. Prepare the test antibody at 2x the desired final concentrations in Thaw Medium 1 (50 µl/well).
2. Prepare a cell suspension of membrane APRIL CHO cells in Thaw Medium 1 at 1.5 x 10^5 cells/ml (50 µl/well).
3. Remove the medium from BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells.
4. Add 50 µl of diluted test antibodies to "Antibody Treated Cells" wells.
5. Add 50 µl of Thaw Medium 1 to the "Untreated Cells" and "Luminescence Background" controls
6. Add 50 µl of Membrane APRIL CHO cells to the "Antibody Treated Cells", and "Untreated Cells" wells.
7. Add 50 µl of Thaw Medium 1 to the "Luminescence Background" control wells (for determining background luminescence).

   Note: If desired, the anti-APRIL antibody can be preincubated with the Membrane APRIL CHO cells for 30 minutes before adding the antibody/APRIL CHO cell suspension to the wells for co-culture.
8. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
9. Add 100 µl of the ONE-Step™ Luciferase reagent per well.
10. Incubate with gentle agitation at RT for ~15 to 30 minutes.
11. Measure luminescence using a luminometer.
12. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB 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.

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

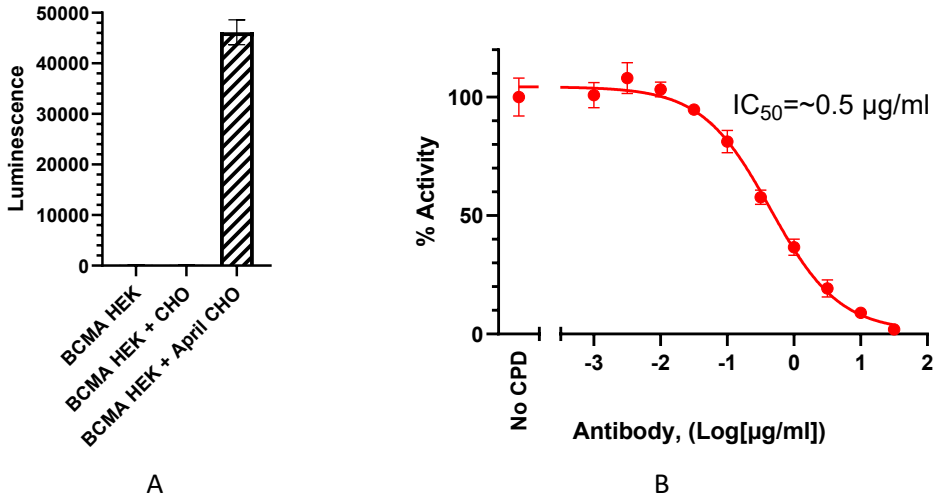

Figure 2: Inhibition of membrane APRIL-induced NF-κB activity by the Anti-APRIL Neutralizing Antibody (Sibeprenlimab), in the BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 Cell Line co-cultured with Membrane APRIL CHO Cell Line.
(A) BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells (#79755) were seeded overnight, and then co-cultured with CHO or Membrane APRIL CHO cells for 5 hours. NF-κB-responsive luciferase activity was measured with ONE-Step™ Luciferase Assay System.
(B) BAFF/APRIL Responsive BCMA-NF-κB Luciferase Reporter HEK293 cells were seeded overnight, and then co-cultured with Membrane APRIL CHO cells for 5 hours, in the presence of various amounts of Anti-APRIL Neutralizing Antibody (Sibeprenlimab). NF-κB-responsive luciferase activity was measured with ONE-Step™ Luciferase Assay System.

Data shown is representative.

References

Lee S.-M., *et al.*, 2010 *Immunology* 131 (3):350-356.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
BAFF/APRIL Responsive TACI-NF-κB Luciferase Reporter Hek293 Cell Line	82791	2 vials
Anti-APRIL Neutralizing Antibody	102736	25 µg/ 100 µg/ 1 mg
BAFF/APRIL Dual Antagonist	102254	25 µg/ 100 µg/ 1 mg

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