Description

CD40L K562 Cell Line is a K562 cell line that expresses full-length human CD40 Ligand (NM_000074.3). This stable clonal cell line was selected for its medium level of CD40L expression compared to the parental K562 cell line. Surface expression of CD40L was confirmed by flow cytometry.

Background

CD40L (CD40 ligand), also known as TNFSF5 (tumor necrosis factor receptor superfamily 5) and CD154, is a type II membrane glycoprotein that exists in cells in a membrane bound (mCD40L) and a soluble (sCD40L) form. It is found at high levels in activated $CD4^+$ T cells, and at lower levels in Th1, Th2, Th17 and Tregs. Expression can also be induced in NK cells, $CD8^+$ T cells, basophils, and others. CD40 and CD40L are stimulatory immune checkpoints, and their signaling is mediated by different TRAF (TNF receptor associated factor), in a cell and stimuli-dependent mode. For example, it mediates the activation of the NF-κB (nuclear factor kappa-B) pathway. The role of CD40 and CD40L as immune checkpoints makes them highly attractive targets in cancer therapy, and several clinical trials using anti-CD40 or anti-CD40L agonist antibodies or trying to increase their expression are underway, targeting both hematological and solid tumors. The inhibition of CD40:CD40L interaction is also clinically relevant, and clinical trials have been focusing on treatment options for lupus, rheumatoid arthritis and ALS (amyotrophic lateral sclerosis). Further studies and development of refined therapies will continue to benefit the cancer therapy field and patients suffering from autoimmune disorders.

Application(s)

- Expansion of CD40 activated B cells from peripheral blood mononuclear cells (PBMCs).
- Screen for anti-CD40L antibodies as therapeutics for autoimmune diseases/transplantation.

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

K562, human lymphoblast cell line, 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.

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 2 (BPS Bioscience #60184):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2M (BPS Bioscience #78181):
RPMI1640 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 or T75 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% CO2 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. Switch to Growth Medium 2M at first and subsequent passages.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, with 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

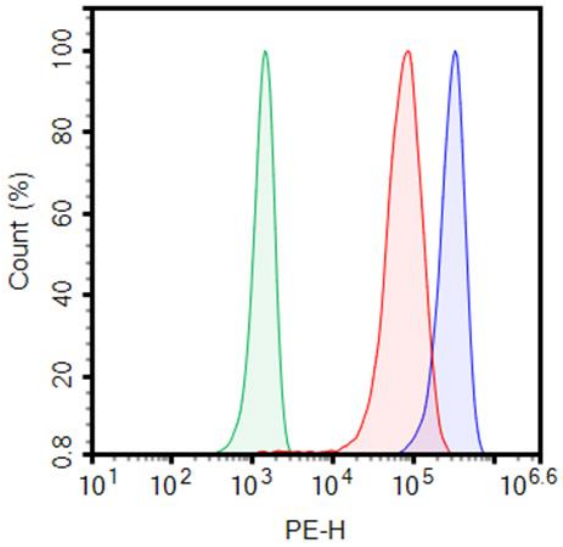

Figure 1: Cell surface expression analysis of CD40L in CD40L K562 Cell Line by flow cytometry. CD40L K562 and control parental K562 cells were stained with PE anti-CD40L Antibody (BioLegend #310806) and analyzed by flow cytometry. Parental K562 cells (green) were compared to K562 cells with medium (red) and high (blue) CD40L expressing levels. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

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

Sequence

Human CD40L sequence (NM_000074.3)
MIETYNQTSPRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKS
QFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTVKRQGLYYIYA
QVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLK
L

References

Annis A., *et al.*, 2004 J. *Amer. Chem. Soc.* 126(4): 15495-15503.
Yan, T., *et al.*, 2001 *J. Cellular Biochem.* 83(2): 320-325.
Tang T., *et al.*, 2021 *Pharmacol Ther* 219:107709.

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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*
CD40 Ligand (CD40L) Lentivirus	78931	500 µl x 2
CD40L (CD154), His-tag (Human) Recombinant	71191	100 µg
CD40:CD40L TR-FRET Assay	79258	384 reactions
CD40 -HEK293 Cell Line	71257	2 vials
CD40-NF-κB Luciferase Reporter Hek293 Cell Line	60626	2 vials
CD40, Fc fusion (Human) Recombinant	71174	25 µg/100 µg

Version 012424