

Description

Recombinant clonal CHO stable cell line constitutively expressing full length human CD14 protein. The surface expression of CD14 in this cell line was validated by flow cytometry.

Background

CD14 (Cluster of Differentiation-14) is a surface antigen expressed predominantly by monocytes & macrophages. It is considered a patterned recognition receptor (PRR), which recognizes pathogen markers. Specifically, CD14 acts as a co-receptor for lipopolysaccharides (LPS) found in the outer membrane of gram-negative bacteria. Through this interaction, CD14 supports initiation of the innate immune response during bacterial infection. CD14 has also been found to contribute toward immune responses to viral pathogens such as human respiratory syncytial virus (RSV), and may amplify the inflammatory response observed in severe cases of SARS-CoV-2 infection (COVID-19). Monoclonal antibodies against CD14 are currently undergoing clinical trial as a candidate treatment to limit severe inflammatory responses common in patients hospitalized with COVID-19.

Application

1. Useful for screening or performing binding assays of antibodies designed against human CD14 in a cellular context.
2. Cell-based assays for the assessment of CD14-mediated cell signaling and/or genetic responses under various experimental conditions (eg. inhibition/neutralization or activation/amplification).

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of 10% DMSO in FBS

Host Cell

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

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539

Storage Conditions



Cells will arrive upon 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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS-validated media, formulation can be found below.



Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3D.

Media Required for Cell Culture

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

Growth Medium 3D (BPS Bioscience #79539): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 3 (**without Geneticin-G418**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**without Geneticin-G418**).
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, add an additional ~ 3 ml of Thaw Medium 3 (**no Geneticin-G418**), and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split.
5. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 3D (**contains Geneticin-G418**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
2. After detachment, add Growth Medium 3D (**contains Geneticin-G418**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 3D and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio is about 1:8 every 3 days.

Cell Freezing

1. To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.

2. After detachment, add Thaw Medium 3 (**no Geneticin-G418**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience, #79796) at $\sim 2 \times 10^6$ cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.



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

Validation Data

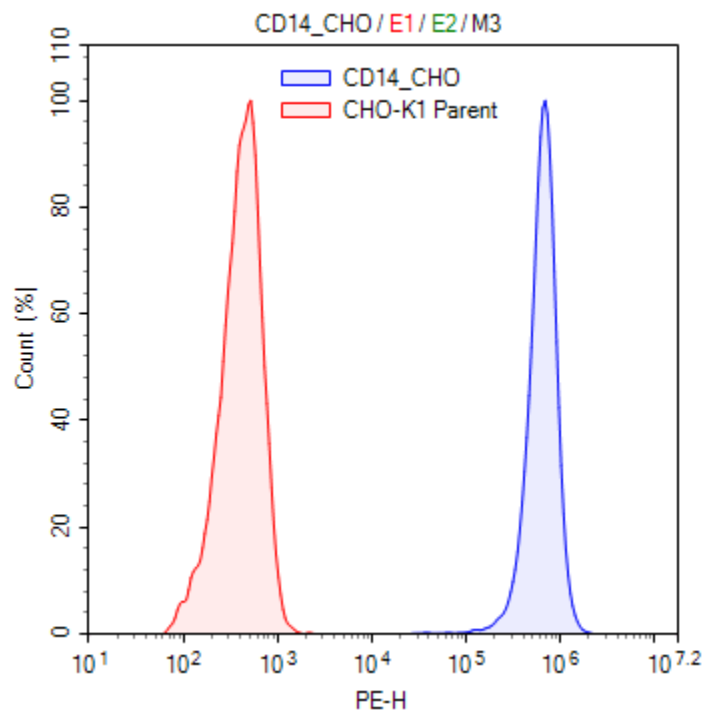


Figure 1. Confirmation of CD14 expression on CHO-K1 recombinant cells

Flow cytometry using PE-conjugated anti-human CD14 antibody (BioLegend, #367104) to detect CD14 surface expression on either the CHO-K1 Recombinant Cell Line (blue) or parental CHO-K1 cells (red).

Sequence

Human CD14 Sequence (Accession Number: NM_000591)

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MERASCLLLLLPLVHVSATTPEPCELDDEDFRCVCNFSEPPQDWSEAFQCVSAVEVEIHAGGLNLEPFLKRVDADADPRQYADT
VKALRVRRRLTVGAAQVPAQLLVGALRVLAYSRLKELTLEDLKITGTMPPLPLEATGLALSSLRLRNVSATGRSWLAELQQWLKP
GLKVLISIAQAHSPAFSCEQVRAFPAALTSLDSDNPGGERGLMAALCPHKFPAIQNLALRNTGMETPTGVCAALAAAGVQPHSL
DLSHNSLRATVNPSPAPRCMWSSALNSLNSLFAGLEQVPKGLPAKLRVLDLSCNRLNRAPQPDELPEVDNLTLDGNPFLVPGTALP
HEGSMNSGVVPACARSTLSVGVSGTLVLLQGARGFA
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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD14, Avi-His-Tag HiP™	101187	100 µg/1 mg
Thaw Medium 3	60186	100 ml/500 ml
Growth Medium 3D	79539	500 ml