Description

The ITGA4/ITGB7 HEK293 Cell Line is a HEK293 cell line expressing the human ITGA4 (integrin α4, also known as CD49d) (NM_000885.6) and ITGB7 (integrin β7) (NM_000889.3) separated by a P2A self-cleaving peptide. This cell line was generated by lentiviral transduction followed by puromycin selection and limiting dilution. Individual clones were screened based on ITGA4/ITGB7 expression by flow cytometry to obtain this ITGA4/ITGB7 expressing cell line.

This cell line was validated by flow cytometry.

Background

Integrins are heterodimeric glycoprotein receptors involved in the regulation of cell adhesion and motility. They are comprised of both an alpha and beta subunit and are classified based upon the ligand motif to which they bind. The Integrin α4β7 (ITGA4/ITGB7) is widely expressed on lymphocytes where it acts as a T cell homing receptor that directs migration to the gut. This process is triggered by the binding of ITGA4/ITGB7 with its ligand, mucosal addressin-cell adhesion molecule 1 (MADCAM-1) which is expressed at high levels in inflamed intestinal tissue. In irritable bowel disease (IBD), ITGA4/ITGB7 is upregulated on activated T cells where it plays an important role in driving chronic inflammation. Integrin blocking therapies have been explored to decrease immune cell homing and subsequent tissue inflammation in irritable bowel diseases including ulcerative colitis (UC) and Crohn's disease (CD), dry eye disease, and multiple sclerosis. Since the ligand for ITGA4/ITGB7, MADCAM-1, is primarily expressed in the intestines, anti-ITGA4/ITGB7 therapies such as Vedolizumab show a gut restricted anti-inflammatory effect without systemic immunosuppression, making them an appealing therapeutic avenue for IBD.

Application

- Screen antibodies targeting ITGA4/ITGB7.

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

HEK293, Human Embryonic Kidney, 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 1	BPS Bioscience #60187
Growth Medium 1N	BPS Bioscience #79801

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 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1N (BPS Bioscience #79801):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and 0.5 µg/ml of Puromycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 1.

   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 1.

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 attachment and viability 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 1N.

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 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N 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 1N.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N and count the cells.

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

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

5. 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

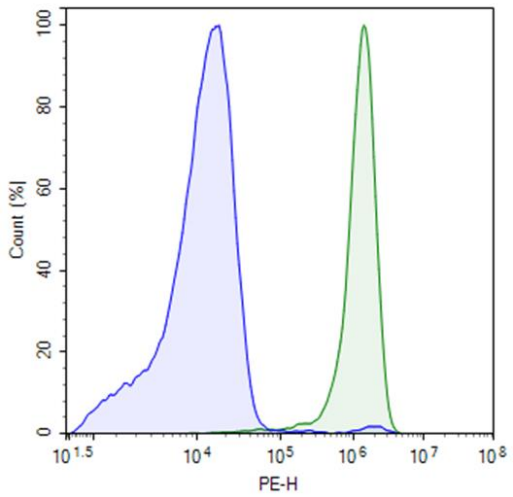

Figure 1: Flow cytometry analysis of ITGA4 cell surface expression in ITGA4/ITGB7 HEK293 Cell Line. ITGA4/ITGB7 HEK293 cells (green) and control parental HEK293 cells (blue) were stained with PE anti-human CD49d Antibody (BioLegend #304304) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

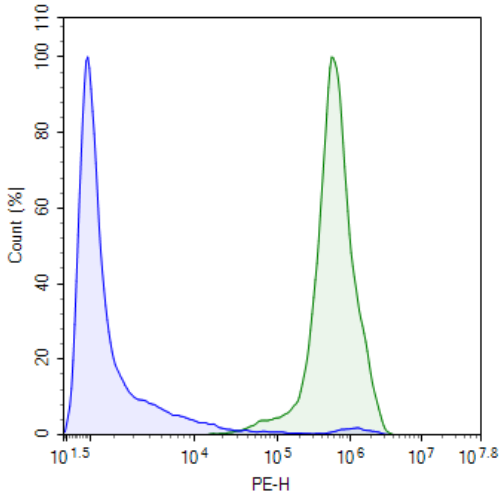

Figure 2: Flow cytometry analysis of ITGB7 cell surface expression in ITGA4/ITGB7 HEK293 Cell Line. ITGA4/ITGB7 HEK293 cells (green) and control parental HEK293 cells (blue) were stained with PE anti-human/mouse Integrin β7 Antibody (BioLegend #321204) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

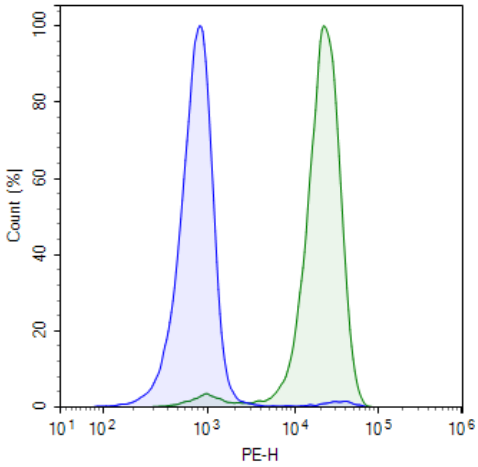

Figure 3: Flow cytometry analysis of ITGA4/ITGB7 cell surface expression in ITGA4/ITGB7 HEK293 Cell Line using Anti-ITGA4-ITGB7 Neutralizing Antibody.
ITGA4/ITGB7 HEK293 cells (green) and control parental HEK293 cells (blue) were stained with Anti-ITGA4-ITGB7 Neutralizing Antibody (#102488) followed by staining with PE Anti-Human IgG Fc (Cedarlane #CL6017PE) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

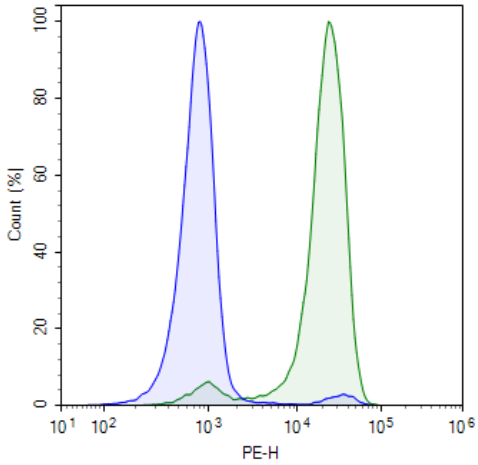

Figure 4: Flow cytometry analysis of ITGA4/ITGB7 integrin cell surface expression in ITGA4/ITGB7 HEK293 Cell Line using Vedolizumab.
ITGA4/ITGB7 HEK293 Cell Line (green) and control parental HEK293 cells (blue) were stained with -Vedolizumab (#83573) followed by staining with PE Anti-Human IgG Fc (Cedarlane #CL6017PE) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

Data shown is representative.

Sequence

Human ITGA4 sequence (accession number NM_000885.6)

MAWEARREPGPRRAAVRETVMLLLCLGVPTGRPYNVDTESALLYQGPHNTLFGYSVVLHSHGANRWLLVGAPTANWLANASV
INPGAIYRCRIGKNPGQTCEQLQLGSPNGEPCGKTCLEERDNQWLGVTLSRQPGENGSIVTCGHRWKNIFYIKNENKLPTGGCYG
VPPDLRTELSKRIAPCYQDYVKKFGENFASCQAGISSFYTKDLIVMGAPGSSYWTGSLFVYNITTNKYKAFLDKQNQVKFGSYLGYS
VGAGHFRSQHTTEVVGGAPQHEQIGKAYIFSIDEKELNILHEMKGKKLGSYFGASVCAVDLNADGFSDLLVGAPMQSTIREEGRV
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SLSMFGQSISGQIDADNNGYVDVAVGAFRSDSAVLLRTRPVVIVDASLSHPESVNRTKFDCVENGWPSVCIDLTLCFSYKGKEVP
GYIVLFYNMSLDVNRKAESPPRFYFSSNGTSDVITGSIQVSSREANCRTHQAFMRKDVRDILTPIQIEAAYHLGPHVISKRSTEEFPP
LQPILQQKKEKDIMKKTINFARFCAHENCSADLQVSAKIGFLKPHENKTYLAVGSMKTLMLNVSLFNAGDDAYETTLHVKLPVGLY
FIKILELEEKQINCEVTDNSGVVQLDCSIGYIYVDHLSRIDISFLLDVSSLSRAEEDLSITVHATCENEEEMDNLKHSRVTVAIPLKYEVK
LTVHGFVNPTSFVYGSNDENEPETCMVEKMNLTFHVINTGNSMAPNVSVEIMVPNSFSPQTDKLFNILDVQTTTGECHFENYQR
VCALEQQKSAMQTLKGIVRFLSKTDKRLLYCIKADPHCLNFLCNFGKMESGKEASVHIQLEGRPSILEMDETSALKFEIRATGFPEP
NPRVIELNKDENVAHVLLEGLHHQRPKRYFTIVIISSSLLLGLIVLLLISYVMWKAGFFKRQYKSILQEENRRDSWSYINSKSNDD

Human ITGB7 sequence (accession number NM_000889.3)

MVALPMVLVLLLVLSRGESELDAKIPSTGDATEWRNPHLSMLGSCQPAPSCQKCILSHPSCAWCKQLNFTASGEAEARRCARRE
ELLARGCPLEELEEPRGQQEVLQDQPLSQGARGEGATQLAPQRVRVTLRPGEPQQLQVRFLRAEGYPVDLYYLMDLSYSMKDDL
ERVRQLGHALLVRLQEVTHSVRIGFGSFVDKTVLPFVSTVPSKLRHPCPTRLERCQSPFSFHHVLSLTGDAQAFEREVGRQSVSGN
LDSPEGGFDAILQAALCQEQIGWRNVSRLLVFTSDDTFHTAGDGKLGGIFMPSDGHCHLDSNGLYSRSTEFDYPSVGQVAQALS
AANIQPIFAVTSAALPVYQELSKLIPKSAVGELSEDSSNVVQLIMDAYNSLSSTVTLEHSSLPPGVHISYESQCEGPEKREGKAEDRG
QCNHVRINQTVTFWVSLQATHCLPEPHLLRLRALGFSEELIVELHTLCDCNCSDTQPQAPHCSDGQGHLQCGVCSCAPGRLGRL
CECSVAELSSPDLESGCRAPNGTGPLCSGKGHCQCGRCSCSGQSSGHLCECDDASCERHEGILCGGFGRCQCGVCHCHANRTGR
ACECSGDMDSCISPEGGLCSGHGRCKCNRCQCLDGYYGALCDQCPGCKTPCERHRDCAECGAFRTGPLATNCSTACAHTNVTL
ALAPILDDGWCKERTLDNQLFFFLVEDDARGTVVLRVRPQEKGADHTQAIVLGCVGGIVAVGLGLVLAYRLSVEIYDRREYSRFEK
EQQQLNWKQDSNPLYKSAITTTINPRFQEADSPTL

References

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Yu Y., *et al*., 2012 *J Cell Biol.* 196(1):131-46.

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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*
Anti-ITGA4-ITGB7 Neutralizing Antibody	102488	25 ug
Vedolizumab	83573	1 mg/ 5 mg/ 10 mg
ITGA4-ITGB7: MAdCAM-1 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	83566	96 reactions
MAdCAM1, Fc-Fusion (IgG1), Avi-Tag, Biotin-Labeled Recombinant	102495-1	25 µg
MAdCAM1, Fc-Fusion (IgG1), Avi-Tag Recombinant	102494-1	25 ug

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