Description

Recombinant CHO-K1 cells expressing cleaved, mature human Mesothelin (MSLN) gene (GenBank Accession #NM_005823.5) under the control of cytomegalovirus (CMV) promoter. This cell line was generated by limited dilution and isolation of individual clones, which were screened based on MSLN expression to obtain a high-expressing cell line.

Background

Mesothelin (MSLN) is a glycophosphatidylinositol (GPI) linked cell-surface protein that is produced as a ~70 kDa precursor protein and cleaved by Furin protease to generate the ~40 kDa mature form. MSLN is frequently over-expressed in mesothelioma, ovarian, pancreatic, and non-small cell lung cancers, while its expression in normal tissues is restricted to the mesothelial lining. MSLN is a tumor-associated antigen and has been an attractive target for targeted immunotherapy approaches, including drug-conjugated antibodies and chimeric antigen receptor T-cells (CAR-T Cells).

Application

Useful as MSLN-expressing target cells in co-culture assays with MSLN CAR-T cells and for screening other MSLN-binding molecules.

Materials Provided

Components	Format
2 vials of frozen cells	2 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Host Cell

CHO-K1 (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 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 3B	BPS Bioscience, #79529

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 components 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. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience, #60186):
Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 3B (BPS Bioscience, #79529):
Thaw Medium 3 and 500 µg/ml of Hygromycin B (Thermo Fisher, #10687010)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to swirl the frozen cells for 30-40 seconds in a 37°C water-bath, then use 1-2 ml Thaw Medium 3 to completely thaw the cells. Transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 3 (**no Hygromycin B**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**no Hygromycin B**).
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. Change medium to fresh Thaw Medium 3 (**no Hygromycin B**), and continue growing culture in a 5% 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 3B (**contains Hygromycin B**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 3B (**contains Hygromycin B**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 3B and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:6 to 1:8 weekly or twice per week.

Cell Freezing

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Thaw Medium 3 (**no Hygromycin B**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Cell Freezing Medium (BPS Bioscience, #79796 or 10% DMSO + 90% FBS) at ~2 x 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

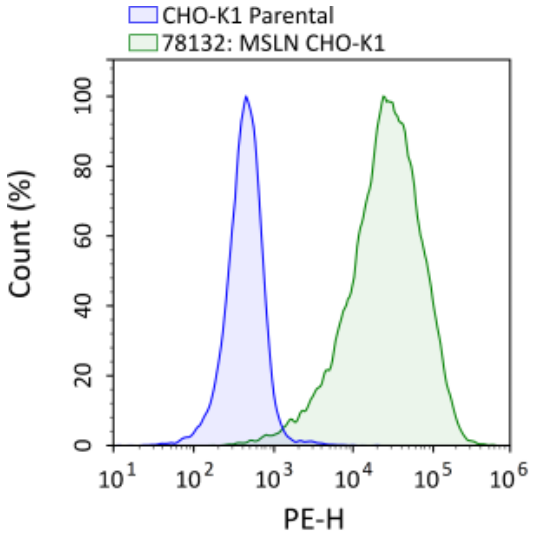

Figure 1. CHO-K1 Parental control or MSLN CHO-K1 cells were analyzed by flow cytometry after staining with PE-labeled Anti-MSLN Antibody (R&D systems, #FAB32652P).

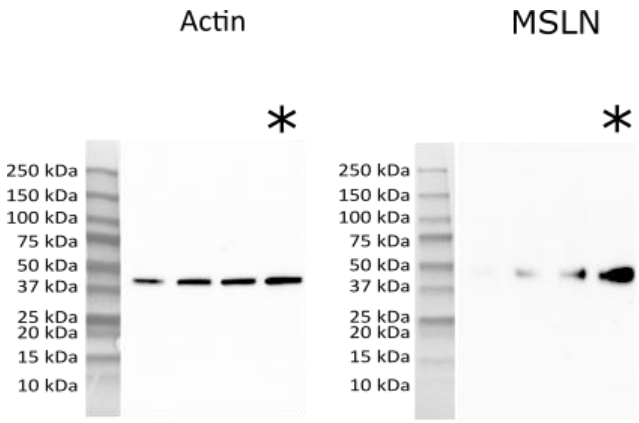

Figure 2. CHO-K1 Parental (Lane 1) or MSLN-Overexpressing Clones (Lanes 2-4) were analyzed by Western Blot using either Rabbit Anti-Actin (Left; Cell Signaling Technology, #4970S) or Rat Anti-MSLN Antibody (Right; R&D systems, #MAB32652) primary antibody. Lane 4 (highlighted by the asterisk) shows the high-expressing MSLN clone selected as BPS Bioscience, #78132. Overexpressed MSLN runs at the molecular weight expected for the cleaved, mature form.

Sequence
Human MSLN sequence (Genbank #NM_005823.5)

MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDGVLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAV
ALAQKNVKLSTEQLRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAALACWGVR
GSLLSEADVRALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRGLLPVLGQ
PIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKTACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPF
TYEQLDVLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGHEMSPQVATLIDRFVKGRGQLDKDTL
DTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNV
SMDLATFMKLRTDAVLPLTVAEVQKLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSMQEALSGT
PCLLGPGPVLTVLALLLASTLA

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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

Product	Cat. #	Size
Thaw Medium 3	60186-1	100 ml
Growth Medium 3B	79529	500 ml
Cell Freezing Medium	79796-1	50 ml

References

1. Asgarov, K., *et al.* (2017). A new anti-mesothelin antibody targets selectively the membrane-associated form. MAbs, **9(3):** 567–577. https://doi.org/10.1080/19420862.2017.1288770

2. Ye, L., *et al.* (2019). Mesothelin-targeted second generation CAR-T cells inhibit growth of mesothelin-expressing tumors *in vivo*. Experimental and Therapeutic Medicine, **17:** 739-747. https://doi.org/10.3892/etm.2018.7015