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Data Sheet

FAP– CHO K1 Recombinant Cell Line

High Expression

Catalog # 79947-H

Background

Fibroblast activation protein alpha (FAP-alpha) belongs to the dipeptidyl peptidase IV (DPP4; CD26) gene family and is a cell-surface serine protease that acts on various hormones and extracellular matrix components. FAP expression under physiological conditions is scarce in healthy adult tissues. But FAP gene is highly upregulated in a wide variety of cancers, and is often used as a marker for pro-tumorigenic stroma. It has also been proposed as a molecular target of cancer therapies, and a great deal of research has gone into design and testing of diverse FAP-targeted treatments.

Description

Recombinant FAP-CHO K1 cell line stably expressing a full length human FAP-alpha protein (Genbank accession No: NM004460.2). Surface expression of FAP was confirmed by flow cytometry. Each stable clonal cell line was selected for different levels of FAP expression (High, Medium, or Low) to mimic different stages of cancer target cells with various FAP expression levels.

Application

- Screen for activators or inhibitors of antibody-mediated signaling for immunotherapy research and drug discovery.
- Characterize FAP antibodies and ligands in binding assays.

Format

Each vial contains ~ 2 x 10⁶ cells in 1 ml of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt.

Culture Medium

Thaw Medium 3 (BPS Bioscience #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 3B (BPS Bioscience #79529): Thaw Medium 3 (BPS Bioscience #60186) plus 500 µg/ml Hygromycin B (Invitrogen, #10687010)

Recommended Culture conditions

Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw medium 3. Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of

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the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 3 (no Hygromycin). Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO₂. The next day, change to fresh Thaw Medium 3 without disturbing the attached cells. Continue to incubate until cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS concentration to 15% for the first week of culture.

Subculture: When cells reach 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml of prewarmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml pre-warmed growth medium. Dispense 1 ml of the cell suspension into a new T75 flask containing pre-warmed 19 ml complete medium (a subcultivation ratio of 1:10 to 1:20 is recommended). Incubate cells in a humidified 37°C incubator with 5% CO₂. To freeze cells, resuspend cell pellet in freezing medium (10% DMSO in FBS).

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Validation

Cell surface expression of human FAP in CHO K1 cells was confirmed by flow cytometry.

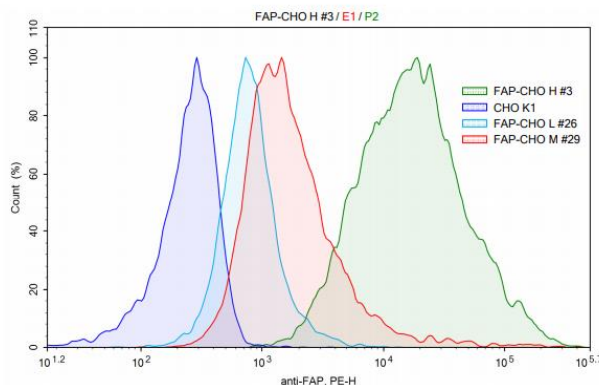


Figure 1. Flow cytometry analysis of cell surface expression of FAP in CHO K1 cells. FAP-CHO K1 cells or control CHO K1 cells were stained with PE-labeled anti-human FAP antibody (R&D Systems #FAB3715P-025) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE. Parental CHO cells (blue); Low expression FAP-CHO cells (turquoise), Medium expression (red), High expression (green).

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Vector and Sequence

Human FAP-alpha (accession number: NM004460.2) was cloned into pCMV3.

MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILNGTFSYKTFPPNWISGQEYLHQS
ADNNIVLYNIETGQSYTILSNRTMKSVNASNYGLSPDRQFVYLESDYSKLRYSYATATYYIYDLNNGEFVR
GNELPRPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIPDWVYEEEMLATKYAL
WWSPNGKFLAYAEFNDDIPVIAYSYYGDEQYPRITINIPYKAGAKNPVVRIFIIDTTYPAYVGPQEVVPA
MIASSDYFWSLWVTDERVCLQWLKRVQNVSVLSICDFREDWQWTWDCPKTQEHIEESRTGWAGGFFV
STPVFSYDAISYYKIFSDKDGKHYKHIYKDTVENAIQITSGKWEAINIFRVTQDSLFFYSNEFEEYPGRRNIY
RISIGSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYGGPISTLHDGRTDQEIKILEENKELENA
LKNIQLPKKEIKLEVDEITLWYKMLPPQFDRSKKYPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVI
ALVDGRGTAFQGDKLLYAVYRKLGVYEVEDQITAVRKFIEGMGFIDEKRIAIWGWWSYGGYVSSLALASGTGL
FKCGIAPVSSWEYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYFRNVVDYLLIHGTADDNVHFQN
SAQIAKALVNAQVDFQAMWYSQNHGLSGLSTNHLYTHMTHFLKQCFSLSD

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Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Thaw Medium 3	60186	100ml
Complete Growth Medium 3B	79529	500ml
FAP-CHO Recombinant Cell Line (Medium Expression)	79947-M	2 vials
FAP-CHO Recombinant Cell Line (Low Expression)	79947-L	2 vials
FAP-CHO Recombinant Cell Line (High Expression)	79947-H	2 vials
FAP Assay Kit	80210	96 rxns
FAP, His-tag (Human)	80100	25 µg
FAP, His-tag (Mouse)	100226	25 µg

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