Cell name: A-431 x ABCB1-Neo M3 (low) / ABCG2
Cat. No. A-0371

Product description

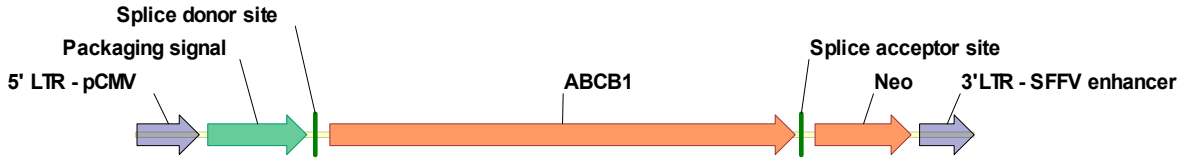
A-431 x ABCB1-Neo M3 (low) / ABCG2 was established by retroviral transduction of human ATP-binding cassette, sub-family G, member 2 (ABCG2) cDNA-containing retrovirus into A-431 x ABCB1-Neo M3 (low) cells and flow cytometry sorting of the ABCG2-immunolabeled population.

A-431 x ABCB1-Neo (low), clone M3 (Cat.No. A-0361), was established by retroviral transduction of human ABCB1 (also known as P-glycoprotein or MDR1) cDNA into A-431 cells and limiting dilution cloning. The clone M3 exhibits relatively low ABCB1 transport activity based on calcein uptake assay.

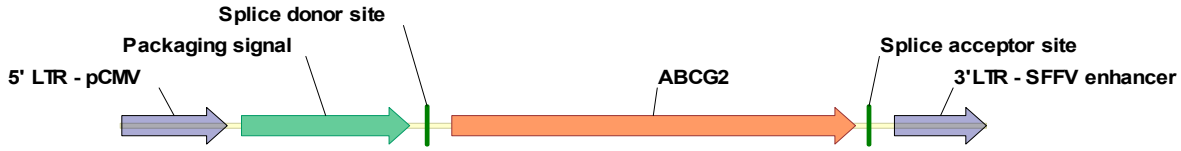
Transgene construct information

Expression cassette map

ABCB1-Neo



ABCG2



Expression cassette features

Element	Type	Species	RefSeq	Mutation / Discrepancy
ABCB1-Neo				
SFFV	promoter, viral, constitutive	-	-	-
ABCB1	CDS	Homo sapiens	AF016535	none / none
Neomycin resistance gene	CDS	-	-	none / none
ABCG2				
SFFV	promoter, viral, constitutive	-	-	-
ABCG2	CDS	Homo sapiens	NM_004827.2	none / none

Transgene protein information

ABCB1-Neo

The transmembrane protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MDR/TAP subfamily. Members of the MDR/TAP subfamily are involved in multidrug resistance. The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multi drug-resistant cells and often mediates the development of resistance to anticancer drugs. This protein also functions as a transporter in the blood-brain barrier. [Source: NCBI Gene, http://www.ncbi.nlm.nih.gov/gene/5243]

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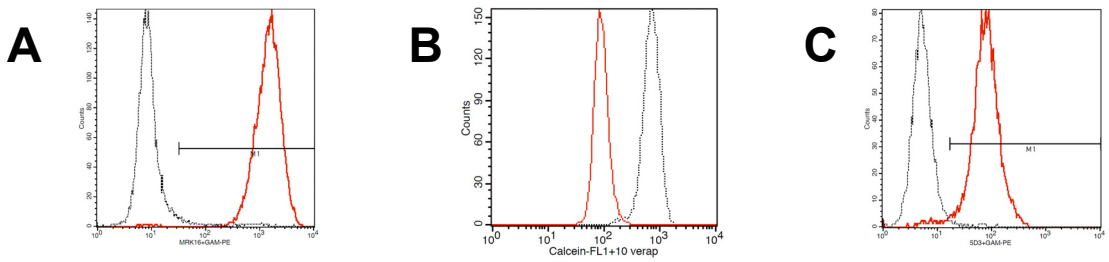
ABCG2

The transmembrane protein encoded by this gene is included in the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the White subfamily. Alternatively referred to as a breast cancer resistance protein, this protein functions as a xenobiotic transporter which may play a major role in multi-drug resistance. It likely serves as a cellular defense mechanism in response to mitoxantrone and anthracycline exposure. Significant expression of this protein has been observed in the placenta, which may suggest a potential role for this molecule in placenta tissue. Multiple transcript variants encoding different isoforms have been found for this gene. [Source: NCBI Gene, http://www.ncbi.nlm.nih.gov/gene/9429]

Cell culture characteristics

Source	Human skin epidermoid carcinoma
Morphology	Epithelial
Growth properties	Adherent
Culture conditions	Culture cells at 37°C in humidified atmosphere with 5% CO_2. The base medium for this cell line is α-MEM with 4.5 g/l glucose. To make the complete growth medium, add fetal bovine serum to a final concentration of 10%.
Subculturing	Remove culture medium, rinse twice thoroughly with PBS, and detach cells by treating with 0.04 mL/cm^2 of 0.25% trypsin / 1 mM EDTA solution for 15 minutes at 37°C. Add 5x volume of complete medium to neutralize trypsin-EDTA, pellet cells at 250 x *g*, discard supernatant, and resuspend in culture medium. Subcultivate in a ratio of 1:4-1:6 three times a week. Recommended plating density: 3x10^4 cells/cm^2.
Preservation	Freeze Medium: Complete growth medium with 10% DMSO. Storage Temperature: Liquid nitrogen vapour phase.
Population doubling time	~30 hours
Sterility testing	Mycoplasma: negative
Comment	This cell line overexpresses Epidermal Growth Factor Receptor (EGFR).

Validation results



A: Red line: A-431 x ABCB1-Neo M3 / ABCG2 cells incubated with anti-Human ABCB1 antibody.
Dotted line: A-431 x ABCB1-Neo M3 / ABCG2 cells incubated with isotype control.
Indirect immunofluorescent labeling with anti-Human ABCB1 antibody (clone MRK16, Alexis Biochemicals / Enzo Life Sciences) or isotype control + PE labeled anti-Mouse IgG secondary antibody.
Detection by flow cytometry.

B: Red line: A-431 x ABCB1-Neo M3 / ABCG cells incubated with calcein.
Dotted line: A-431 x ABCB1-Neo M3 / ABCG cells incubated with calcein and verapamil.
Calcein uptake was detected by flow cytometry at 485 nm in the presence or absence of verapamil, an inhibitor of ABCB1.

C: Red line: A-431 x ABCB1-Neo M3 / ABCG2 cells incubated with anti-Human ABCG2 antibody.
Dotted line: A-431 x ABCB1-Neo M3 / ABCG2 cells incubated with isotype control.
Indirect immunofluorescent labeling with anti-Human ABCG2 (clone 5D3) antibody (R&D Systems®) or isotype control + PE labeled anti-Mouse IgG secondary antibody. Detection by flow cytometry.

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