Cell name: A-431 x ABCB1-Neo M13 (high)
Cat. No. A-0360

Product description

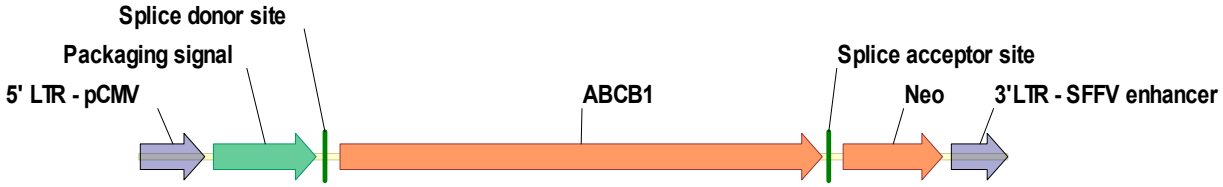
A-431 x ABCB1-Neo, clone M13, was established by retroviral transduction of human ABCB1 (also known as P-glycoprotein or MDR1) cDNA into A-431 cells and limiting dilution cloning. The clone M13 exhibits relatively high ABCB1 transport activity based on calcein uptake assay.

Transgene construct information

Expression cassette type

Single-promoter, bicistronic

Expression cassette map



Expression cassette features

Element	Type	Species	RefSeq	Mutation / Discrepancy
SFFV	promoter, viral, constitutive	-	-	-
Splice donor site	Splice donor site	-	-	-
ABCB1	CDS	Homo sapiens	AF016535	none / none
Splice acceptor site	Splice acceptor site	-	-	-
Neomycin resistance gene	CDS	-	-	none / none

Transgene protein information

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 multidrug-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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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.

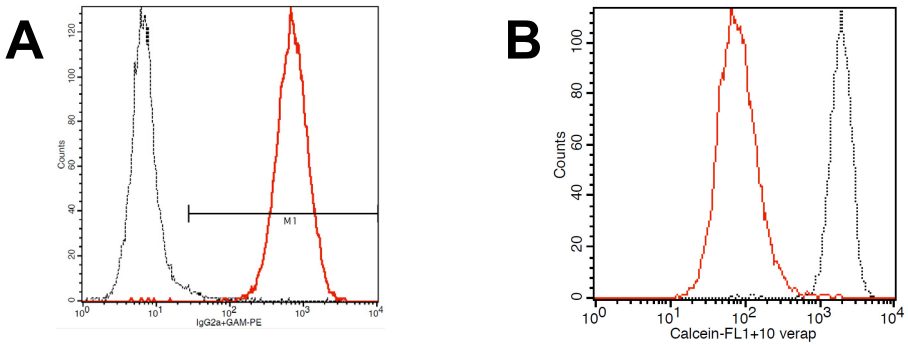
Preservation
Freeze Medium: Complete growth medium with 10% DMSO.
Storage Temperature: Liquid nitrogen vapour phase.

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 M13 cells incubated with anti-Human ABCB1 antibody.
Dotted line: A-431 x ABCB1-Neo M13 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 M13 cells incubated with calcein.
Dotted line: A-431 x ABCB1-Neo M13 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.