Cell name: A-431 x ABCG2(K86M)-Neo
Cat. No. A-0320

Product description

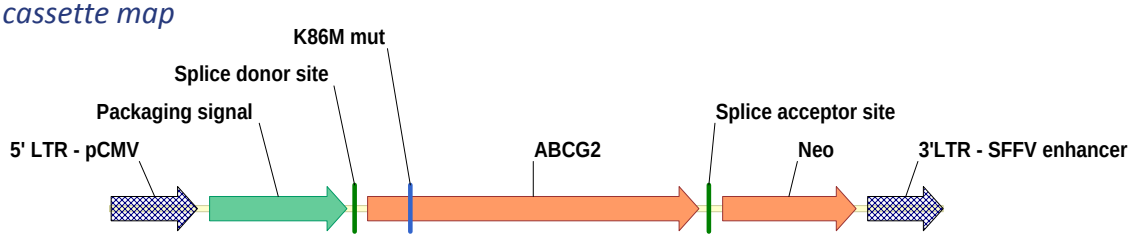
A-431 x ABCG2(K86M)-Neo was established by retroviral transduction of the inactive catalytic center mutant K86M of human ABCG2 (ATP-binding cassette, sub-family G, member 2) cDNA, along with the Neomycin resistance gene, into A-431 cells and flow cytometry sorting of the anti-Human ABCG2 immunolabeled population.

Transgene construct information

Expression cassette type

Single-promoter, monocistronic

Expression cassette map



Expression cassette features

Element	Type	Species	RefSeq	Mutation / Discrepancy
SFFV	promoter, viral, constitutive	-	-	-
Splice donor site	Splice donor site	-	-	-
ABCG2	CDS	Homo sapiens	NM_004827.2	K86M / none
Splice acceptor site	Splice acceptor site	-	-	-
Neomycin resistance gene	CDS	-	-	none / none

Transgene and mutation information

Wild-tpye ABCG2, a member of the ABC transmembrane transporter family, 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.
The K86M mutation affects the catalytic center in the Walker A motif of ABCG2 and causes loss of both transport and ATPase activities.

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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.

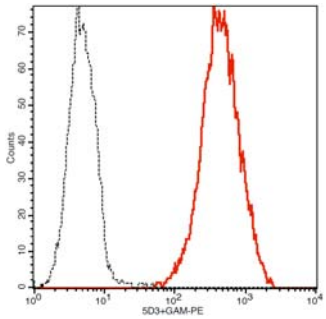
Population doubling time
~30 hours

Sterility testing
Mycoplasma: negative

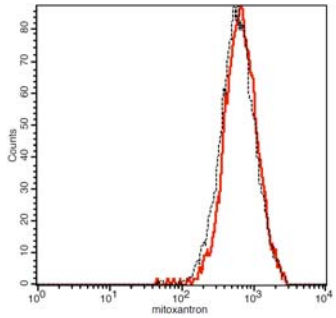
Comment
This cell line overexpresses Epidermal Growth Factor Receptor (EGFR).

Validation results

A



B



A: Red line: A-431 x ABCG2(K86M)-Neo cells incubated with anti-Human ABCG2 antibody.
Dotted line: A-431 x ABCG2(K86M)-Neo 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 IgG2b secondary antibody. Detection by flow cytometry.

B: Red line: A-431 x ABCG2(K86M)-Neo cells incubated with mitoxantrone.
Dotted line: A-431 x ABCG2(K86M)-Neo cells incubated with mitoxantrone and Ko143.
Mitoxantrone uptake detected by flow cytometry at 633 nm in the presence or absence of Ko143, a specific inhibitor of ABCG2.