

Cell name: A-431 x ABCG2(Q141K)-Neo
Cat. No. A-0330

Product description

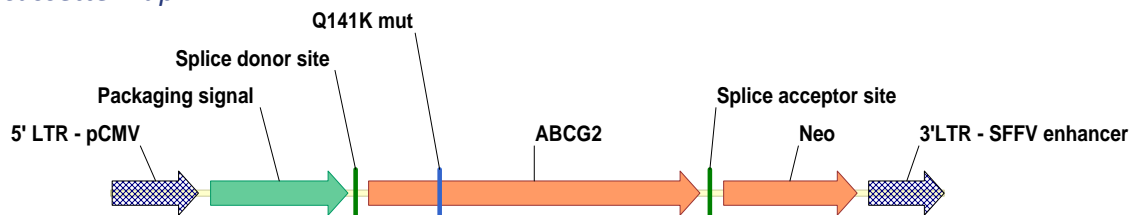
A-431 x ABCG2(Q141K)-Neo was established by retroviral transduction of the hypomorphic mutant Q141K of human ABCG2 (ATP-binding cassette, sub-family G, member 2) cDNA, along with the Neomycin resistance gene, into A-431 cells and flow cytometry sorting of the anti-Human ABCG2 (clone 5D3) immunolabeled 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	Q141K / none
Splice acceptor site	Splice acceptor site	-	-	-
Neomycin resistance gene	CDS	-	-	none / none

Transgene protein information

Wild-type 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 Q141K mutation is a common reduced functionality polymorphic variant of ABCG2. Since ABCG2 also functions as a urate transporter, hypomorphic variants such as Q141K are significantly associated with gout (Woodward et al. Proc Natl Acad Sci U S A. 2009;106:10338-42.)

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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% CO₂. 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/cm² 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: 3x10⁴ cells/cm².

Preservation

Freeze Medium: Complete growth medium with 10% DMSO.

Storage Temperature: Liquid nitrogen vapour phase.

Population doubling time

~30 hours

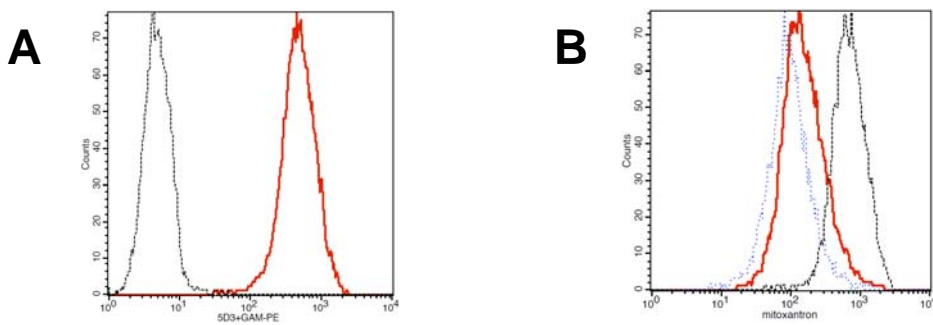
Sterility testing

Mycoplasma: negative

Comment

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

Validation results



A: Red line: A-431 x ABCG2(Q141K)-Neo cells incubated with anti-Human ABCG2 antibody.

Dotted line: A-431 x ABCG2(Q141K)-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(Q141K)-Neo cells incubated with mitoxantrone.

Dotted blue line: A-431 x ABCG2 (wild type) cells incubated with mitoxantrone.

Dotted gray line: A-431 x ABCG2(Q141K)-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.

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