

Cell name: HEK293 x KCNQ1(E261K)-GFP Cat. No. H-0263

Product description

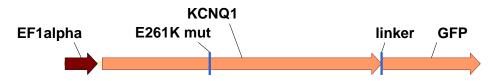
HEK293 x KCNQ1(E261K)-GFP was established by lentiviral transduction of GFP-tagged missense mutant KCNQ1 potassium channel into HEK293 cells and flow cytometry sorting of the GFP-positive population.

Transgene construct information

Expression cassette type

Single-promoter, monocistronic, fusion

Expression cassette map



Expression cassette features

Element	Туре	Species	RefSeq	Mutation / Discrepancy
EF1α	promoter, eukaryotic, constitutive	-	-	-
KCNQ1	CDS	Homo sapiens	NM 000218.2	E261K / stop codon altered to TCC
linker	linker	-	-	-
GFP	CDS	-	-	none / none

Transgene protein information

The wild-type *KCNQ1* gene encodes a voltage-gated potassium channel required for repolarization phase of the cardiac action potential. This protein can form heteromultimers with two other potassium channel proteins, KCNE1 and KCNE3. Mutations in this gene, including the E261K missense mutation, are associated with hereditary long QT syndrome 1 (also known as Romano-Ward syndrome). [Modified after: NCBI Gene, http://www.ncbi.nlm.nih.gov/gene/3784]

The C-terminal GFP fusion of KCNQ1(E261K) was created by the laboratory of A. Tinker (Wilson et al. Cardiovasc Res. 2005;67:476-86.) GFP is a naturally occurring fluorescent protein originally isolated from the jellyfish *Aequorea victoria*. Since its discovery, numerous variants with altered excitation and emission properties have been engineered. The GFP variant used herewith is most efficiently excited around 510 nm and emits at 527 nm.

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Mutation information

E261K is a loss-of-function mutation of the KCNQ1 channel associated with hereditary long QT syndrome. [Reference: Franqueza et al. J Biol Chem. 1999 Jul 23;274(30):21063-70.]

Cell culture characteristics

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 D-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 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 1:10. Recommended plating density: $4x10^4$ cell/cm².

Preservation

Freeze Medium: Complete growth medium with 10% DMSO.

Storage Temperature: Liquid nitrogen vapour phase.

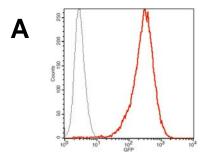
Population doubling time

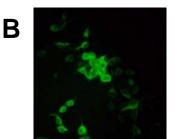
~24 hours

Sterility testing

Mycoplasma: negative

Validation results





A: Red line: HEK293 x KCNQ1(E261K)-GFP cells.

Dotted line: HEK293 cells.

Detection of GFP by flow cytometry.

B: Detection of KCNQ1(E261K)-GFP in HEK293 x KCNQ1(E261K)-GFP cells by confocal fluorescence microscopy.

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