Ion Channel stable cell line manual

Catalog Number	Product	Amount
SC022-RB	HEK293-CFTR cell line (**chloride** channel) with RFP and Blasticidin dual marker	1 vial of cells (2×10^6 cells) in 80% DMEM, 10% FBS, 10% DMSO
SC023-RB	HEK293-CLCN2 cell line (**chloride** channel) with RFP and Blasticidin dual marker	
SC024-RB	HEK293-TRPC3 cell line (**calcium** channel) with RFP and Blasticidin dual marker	
SC025-RB	HEK293-KCNN4 cell line (**potassium** channel) with RFP and Blasticidin dual marker	
SC026-RB	HEK293-ATP2B2 cell line (**calcium** channel) with RFP and Blasticidin dual marker	
SC027-RB	HEK293-TRPV1 cell line (**non-selective cation** channel) with RFP and Blasticidin dual marker	

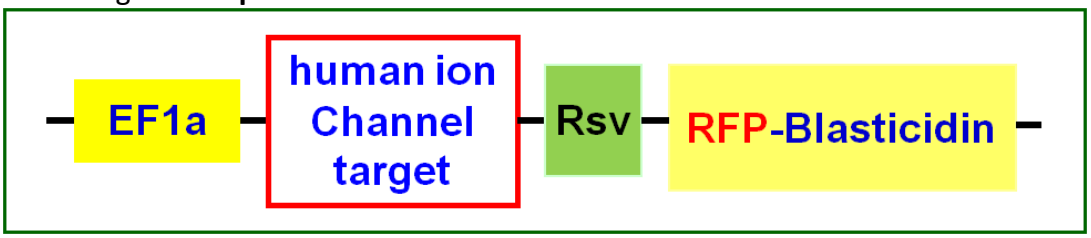
Storage: store in liquid nitrogen

Product Description

The HEK293 Cell Line is a stable line established from primary embryonic human kidney cells transformed with sheared human adenovirus type 5 DNA. The expressed E1A adenovirus gene allows these cells to produce very high levels of protein.

The human ion-channel stable cell lines are derived from the adhesive enhanced HEK293 cells, transformed by the expression lentivirus expressing a well characterized human ion-channel target. The expression cassette (see the **structure scheme** below) was stably integrated into HEK293 cells. Each human ion-channel target codon sequence is natively expressed (without any tags) under an enhanced EF1a promoter. The cell lines also have a **RFP-blasticidin**, (fluorescent-antibiotic) fusion dual marker under RSV promoter. Therefore, **each cell also demonstrates RFP signal** which can be visualized under fluorescent microscope. Each target genomic integration was verified by gPCR, and the target expression was also validated by Western blot.

The integrated **expression cassette** in HEK293 cell:



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1. **Ion-channel targets**:

   The codon sequence of each human ion-channel gene is natively expressed in each cell line. All target sequences were fully verified (**target ID** for the sub-cloned codon sequences is identical to the the CDS region sequences in NCBI database).

Catalog #	Target ID	Target Name
SC022-RB	**NM_000492**	CFTR (cystic fibrosis transmembrane conductance regulator)
SC023-RB	**NM_004366**	CLCN2 (chloride channel, voltage-sensitive 2)
SC024-RB	**NM_003305**	TRPC3 (transient receptor potential cation channel, subfamily C, member 3)
SC025-RB	**NM_002250.2**	KCNN4 (potassium intermediate/small conductance calcium-activated channel, subfamily N)
SC026-RB	**NM_001001331.2.**	ATP2B2 (ATPase, Ca++ transporting, plasma membrane 2)
SC027-RB	**NM_080704.3**	TRPV1 (transient receptor potential cation channel, subfamily V, member 1

2. Ion-Channel target information:

   - **CFTR**:

     This gene encodes a protein involved in multi-drug resistance. It belongs to the ATP-binding cassette (ABC) subfamily. The ABC proteins transport various molecules across extra- and intra-cellular membranes. The CFTR functions as a chloride channel and controls the regulation of other transport pathways. It has been linked to chronic bronchopulmonary disease (with recurrent respiratory infections), pancreatic insufficiency (which leads to malabsorption and growth retardation) and elevated sweat electrolytes. Mutations in this gene are associated with the autosomal recessive disorders cystic fibrosis and congenital bilateral aplasia of the vas deferens.

   - **CLCN2**:

     This gene encodes a voltage-gated chloride channel. The encoded protein is a transmembrane protein that maintains chloride ion homeostasis in various cells. Defects in this gene may be a cause of certain epilepsies.

   - **TRPC3**:

     The protein encoded by this gene is a membrane protein that can form a non-selective channel permeable to calcium and other cations. The encoded protein appears to be induced to form channels by a receptor tyrosine kinase-activated phosphatidylinositol second messenger system and also by depletion of intracellular calcium levels.

- **KCNN4:**
  The protein encoded by this gene is part of a potentially heterotetrameric voltage-independent potassium channel that is activated by intracellular calcium. Activation is followed by membrane hyperpolarization, which promotes calcium influx. The encoded protein may be part of the predominant calcium-activated potassium channel in T-lymphocytes. This gene is similar to other KCNN family potassium channel genes, but it differs enough to possibly be considered as part of a new subfamily.

- **ATP2B2:**
  The protein belongs to the family of P-type primary ion transport ATPases characterized by the formation of an aspartyl phosphate intermediate during the reaction cycle. These enzymes remove bivalent calcium ions from eukaryotic cells against very large concentration gradients and play a critical role in intracellular calcium homeostasis.

- **TRPV1:**
  Capsaicin, the main pungent ingredient in hot chili peppers, elicits a sensation of burning pain by selectively activating sensory neurons that convey information about noxious stimuli to the central nervous system. The protein encoded is a receptor for capsaicin and is a non-selective cation channel that is structurally related to members of the TRP family of ion channels. This receptor is also activated by increases in temperature in the noxious range, suggesting that it functions as a transducer of painful thermal stimuli in vivo.

Culture procedures

1. Thaw the frozen vial of cells quickly in a 37°C water bath (1-3min), decontaminate the outside of the vial with 70% ethanol.
2. Transfer the entire content of the cryovial into a T-75 cm^2 flask containing 15 ml of pre-warmed complete medium. Incubate the cells overnight in a 37°C incubator, 5% CO_2.
3. The following day, replace the medium with 15 ml of pre-warmed, **complete medium (**see below for its components).
   Optional: No need to add antibiotic. But if maintenance of cell line's genetic stability for the long-term culture is desired, add blasticidin antibiotic at final concentration of 10 ug/ml into the medium.
4. Incubate the cells and monitor cell density.
5. Pass cells (1:10 dilution) when the culture reaches 80-90% confluency.
6. Freeze cells at a density of 3 x 10^6 cells/ml using 90% complete medium with 10% DMSO.

Complete medium

D-MEM (high glucose)
2mM L-glutamine
10% Fetal Bovine Serum (FBS)
0.1 mM MEM Non-Essential Amino Acids (NEAA)
1% Pen-strep

Quality Control

Each vial contains more than 2 x 10^6 cells with >95% viability before freezing. Cells are tested free of bacteria, viruses, mycoplasma.

Warranty and user terms

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Related Products:

Pre-made stable cell lines:

Catalog #	Product Name
SC001	HEK293-GFP stable cell line
SC002-Bsd	luciferase (firefly), HEK293 stable cell line (Blasticidin)
SC002-GB	luciferase (firefly), HEK293 stable cell line (GFP-Blasticidin)

SC002-GP	luciferase (firefly), HEK293 stable cell line (GFP-Puromycin)
SC002-Neo	luciferase (firefly), HEK293 stable cell line (Neomycin)
SC002-Puro	luciferase (firefly), HEK293 stable cell line (Puromycin)
SC002-RB	luciferase (firefly), HEK293 stable cell line (RFP, Blasticidin)
SC002-RP	luciferase (firefly), HEK293 stable cell line (RFP-Puromycin)
SC003	LacZ stable cell line
SC004-Bsd	CRE stable cell line with blasticidin marker
SC004-GP	CRE stable cell line with GFP-Puromycin dual marker
SC004-Neo	CRE stable cell line with neomycin marker
SC004-Puro	CRE stable cell line with Puromycin marker
SC004-RB	CRE stable cell line with RFP-blasticidin dual marker
SC004-RP	CRE stable cell line with RFP-Puromycin dual marker
SC005-Bsd	HEK293-TetR (Bsd)
SC005-GB	HEK293-TetR (GFP-Bsd)
SC005-Hygro	HEK293-TetR (Hygro)
SC005-Neo	HEK293-TetR (Neo)
SC005-Puro	HEK293-TetR (Puro)
SC005-RB	HEK293-TetR (RFP-Bsd)
SC005-RP	HEK293-TetR (RFP-Puro)
SC006	Flp stable cell line
SC007	HEK293-RFP stable cell line
SC008	GFP-LacZ & RFP stable cell line
SC009	GFP & RFP HEK293 stable cell line
SC010	HEK293-CFP stable cell line
SC011	HEK293-YFP stable cell line
SC012	TAT stable cell line in HEK293
SC013	Glutamine Synthesis stable cell line
SC014	Inducible h P53 stable cell line

SC015	h OCT3/4 stable cell line
SC016	h LIN28 stable cell line
SC018-Bsd	Color Switch, CRE report cell line: HEK293-loxP-GFP-RFP (Bsd)
SC018-Neo	Color Switch, CRE report cell line: HEK293-loxP-GFP-RFP Neo)
SC018-Puro	Color Switch, CRE report cell line: HEK293-loxP-GFP-RFP (Puro)
SC020-Puro	luciferase (Renilla), HEK293 stable cell line (Puromycin)
SC020-RP	luciferase (Renilla), HEK293 stable cell line (RFP-Puromycin)
SC021-GB	Luciferase (firefly) and CRE co-expression stable cell line (GFP-Blasticidin)
SC021-Puro	Luciferase (firefly) and CRE co-expression stable cell line (puromycin)
SC028	Inducible RFP HEK293 stable cell line
SC029	inducible RFP HEK293 stable cell line with GFP marker
SC030	inducible GFP HEK293 stable cell line with RFP marker
SC031-Puro	Hela-RFP stable cell line
SC032-Bsd	Luciferase (firefly), Hela stable cell line (Blasticidin)
SC032-GB	Luciferase-2A-GFP, Hela stable cell line (Blasticidin)
SC032-GN	Luciferase-2A-GFP, Hela stable cell line (Neomycin)
SC032-GP	Luciferase-2A-GFP, Hela stable cell line (Puromycin)
SC032-Puro	Luciferase (firefly), Hela stable cell line (Puromycin)
SC032-RB	Luciferase-2A-RFP, Hela stable cell line (Blasticidin)
SC032-RN	Luciferase-2A-RFP, Hela stable cell line (Neomycin)
SC032-RP	Luciferase-2A-RFP, Hela stable cell line (Puromycin)
SC033	Inducible GFP HEK293 stable cell line
SC034-Puro	Hela-GFP stable cell line
SC035-Puro	Hela-TetR (Puro) stable cell line