B'SYS GmbH

CHO K_{Ca}3.1 (SK4 / IK1) Cell Line

Specification Sheet

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1 PRODUCT SHIPMENT

1.1 Product Format

CHO cells stably transfected with recombinant K_{Ca}3.1 potassium channel:

- 0.75 mL aliquots of frozen cells at 1 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO
- Cells are frozen at passage number, see vial

1.2 Mycoplasma Certificate

B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

1.3 Assays

CHO K_{Ca}3.1 cells were validated for manual patch-clamping

2 INTRODUCTION

K_{Ca} channels differ in their primary amino acid sequences and exhibit different single-channel conductances and pharmacological profiles. Each subtype plays a specific role in several physiological processes, including neurosecretion, smooth muscle tone, action potential shape and spike frequency adaptation.
One group of calcium activated potassium channels include ion channels with intermediate conductance: K_{Ca}3.1 (IK). These channels are voltage-insensitive and are activated by low concentrations of internal calcium (less than 1.0 µM). Both IK and SK channels play roles in processes involving calcium-dependent signalling in both electrically excitable and non excitable cells. Unless they do not bind calcium directly they detect it by virtue of calmodulin, which is constitutively bound to the C-terminal region. Binding of calcium to this calmodulin results in conformational changes that are in turn responsible for channel gating.

3 CELL CULTURE CONDITIONS

3.1 General

CHO K_{Ca}3.1 cells are incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing F12 (HAM) medium supplemented with 10% foetal bovine serum, 1.0% Penicillin/Streptomycin solution and 100 µg/mL Hygromycin. The CHO K_{Ca}3.1 cells are passaged at a confluence of about 80%. For electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes containing complete medium.

- All solutions and equipment coming in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.
- Be sure to have frozen cell stocks at hand before starting experiments.
- Cells should be split every 2-3 days at 70% - 80% confluence at 1:3 to 1:5 ratio.

Table 1: Cell culture reagents

Product	Supplier	Order number
Nutrient mixture F-12 Ham (F12 Ham)	Sigma-Aldrich	N6658
Fetal Bovine Serine (FBS)	Gibco	10270-106
Penicillin / Streptomycin (100x)	Gibco	10378-016
Phosphate Buffered Saline (PBS, without Ca^{2+} and Mg^{2+})	Sigma-Aldrich	D8537
Hygromycin B (50 mg/mL)	Gibco	10687010
Detachin	Genlantis	T100T100
Trypsin EDTA (10x)	Sigma-Aldrich	T4174
DMSO	Sigma-Aldrich	D2438

For the preparation of 1X Trypsin/EDTA, the 10X solution is diluted in PBS (without Ca^{2+} and Mg^{2+}), aliquoted and stored in the freezer.

3.2 Recommended Complete Medium

- F12 (HAM) with L-Glutamine
- 10% FBS
- 1.0% Penicillin/Streptomycin

3.3 Antibiotics

- CHO K_{Ca}3.1 clones were selected under 500 µg/mL Hygromycin antibiotic pressure.
- To cultivate CHO K_{Ca}3.1 cells, a reduced antibiotic pressure (100 µg/mL) must be used.
- To separate CHO K_{Ca}3.1 cells from non-transfected cells, use 500µg/mL Hygromycin.

Remark: The permanent application of high antibiotic pressure has no effect on current density.

3.4 Thawing Cells

- Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
- Decontaminate outside of vial with 70% Ethanol.
- Transfer cells to a T-25 culture flask containing 5 mL complete medium

- Incubate cells at 37°C for at least 4-6 hours to allow the cells to attach to the bottom of the flask
- Once cells attach to the bottom of the flask and look healthy, aspirate off the medium and replace with 5 mL complete medium containing selection antibiotics for cultivation (see 3.3)
- To check whether cells are attached properly, the flask can be gently moved while looking under the microscope
- If 48h after thawing the confluency is below 50%, replace the medium in the flask with fresh medium containing antibiotics
- Incubate cells at 37°C and check them daily until 50% to 80% confluency is reached.

3.5 Splitting Cells

- When cells are 50% to 80% confluent remove complete medium.
- Wash cells with 1xPBS to remove excess medium
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C.
- Detach cells by gently tapping the sides of the flask add complete medium and pipet up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
- Use remaining suspension for counting the cells.

3.6 Freezing Medium

- Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.
- Sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

3.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium and wash cells with 1xPBS.
- Add 0.5 mL to 1 mL Detachin (or 1x Trypsin/EDTA) and incubate for 2 min at 37°C
- Detach cells by gently tapping the sides of the flask, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells at 200 g using a centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 1.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.75 mL of cell suspension into each cryovial.
- Overnight incubate cells in a polystyrene box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

3.8 Stability of CHO K_{Ca}3.1 cells

CHO K_{Ca}3.1 cells stably express functionally active K_{Ca}3.1 potassium channels over 20 passages. Under recommended cell culture conditions, no variation in current density was observed during this time.

4 K_{CA}3.1 (SK4 / IK1) SEQUENCE

4.1 Accession Number NP_002241.1

Cloned cDNA (KCNN4) encodes for the protein of the K_{Ca}3.1 (SK4 / IK1) channel (NP_002241.1):

MGGDLVLGLGALRRRKRLLEQEKSLAGWALVLAGTGIGLMVLHAEMLWFGGCSWALYLFLVKCTISISTFLLLCLIVAFH
AKEVQLFMTDNGLRDWRVALTGRQAAQIVLELVVCGLHPAPVRGPPCVQDLGAPLTSPQPWPGFLGQGEALLSLAMLLRL
YLVPRAVLLRSGVLLNASYRSIGALNQVRFRHWFVAKLYMNTHPGRLLLGLTLGLWLTTAWVLSVAERQAVNATGHLSDT
LWLIPITFLTIGYGDVVPGTMWGKIVCLCTGVMGVCCTALLVAVVARKLEFNKAEKHVHNFMMDIQYTKEMKESAARVLQ
EAWMFYKHTRRKESHAARRHQRKLLAAINAFRQVRLKHRKLREQVNSMVDISKMHMILYDLQQNLSSSHRALEKQIDTLA
GKLDALTELLSTALGPRQLPEPSQQSK*

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