B'SYS GmbH

HEK 293 GRIN1 Cells

Specification Sheet

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1 BACKGROUND

1.1 The NMDA receptor

The NMDA receptor gates a nonselective cation channel playing a critical role in synaptic plasticity. It consists of NR1 and NR2 subunits forming a a heterotetramer that is activated by application of glutamate (or NMDA) together with glycine. NR1 is coded by GRIN1, NR2 is coded by one of the four isoforms GRIN2A to GRIN2D.

1.2 HEK GRIN1

For analysis of functional NMDA channels, human GRIN1 cDNA was stably transfected into HEK 293 cells. This cell line is optimal to analyze various NR1/NR2 combinations when transiently transfected with cDNA encoding for NR2 subunits.

2 PRODUCT SHIPMENT

2.1 Product Format

HEK cells stably transfected with recombinant human NMDA (GRIN1) channel:

- aliquots of frozen cells at about 1.0 E+06 cells/mL
- Cells are frozen in complete medium with 10% DMSO

2.2 Mycoplasma Certificate

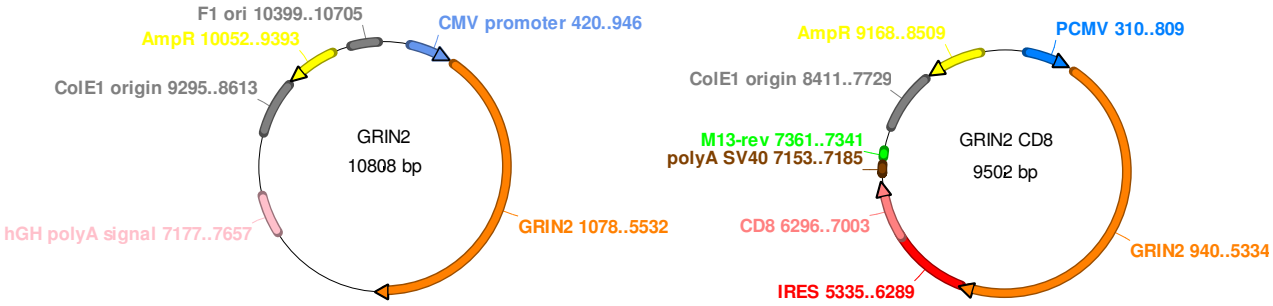
B'SYS periodically tests cells for presence of mycoplasma by means of highly sensitive PCR based assays. All delivered cells are free of mycoplasma.

3 NR2 SUBUNITS

B'SYS offers cDNAs encoding for various NR2 subunits. These cDNAs were successfully used to study NMDA receptors using manual patch-clamping. The plasmids are available with or without the marker gene CD8. The CD8 gene is under control of the same promoter like the NR2 subunit, but separated by an IRES (internal ribosome entry site) element. By using Dynabeads M-45 CD8 (Life Technologies) successfully transfected cells can be identified and /or enriched (Jurman et al. 1994).

Available plasmids, encoding for NR2 subunits:

Subunit	Plasmid without CD8 marker	Plasmid containing CD8 marker
NR2A	+	+
NR2B	+	+
NR2C	-	-
NR2D	+	-



4 CELL CULTURE CONDITIONS

4.1 General

HEK GRIN1 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 DMEM/F12 medium supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin solution, 100 µg/mL Hygromycin and 15 µg/mL Blasticidin. The HEK GRIN1 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% confluency at 1:3 to 1:5 ratio (low confluence reduces cell proliferation).

4.2 Recommended Complete Medium

- DMEM/F12 with GlutaMAX I or L-Glutamine
- 10% FBS
- 1.0% Penicillin/Streptomycin

4.3 Antibiotics

- HEK GRIN1 clones were selected under 500 µg/mL Hygromycin and 15 µg/mL Blasticidin pressure.
- To cultivate HEK GRIN1 cells, also a reduced antibiotic pressure (see 4.3) can be used.
- To separate HEK GRIN1 cells from untransfected cells, use the selection pressure.

Remark: The permanent application of antibiotics has no effect on current density.

4.4 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 4-6 h to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 5 mL complete medium & antibiotics.
- Antibiotics: 100 µg/mL Hygromycin and 15 µg/mL Basticidin.
- Incubate cells and check them daily until 70% - 80% confluence is reached.

4.5 Splitting Cells

- When cells are 80%-90% confluent remove medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA or Detachin and incubate 30 s at room temperature.
- Remove Trypsin/EDTA or Detachin quickly and incubate cells for 2 min at 37°C.
- Detach cells, 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.

4.6 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) or use sterile DMSO

4.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium.
- Wash cells once with 1x PBS to remove excess medium.
- Add 1x Trypsin/EDTA or Detachin and incubate 30 s at room temperature.
- Remove Trypsin/EDTA or Detachin quickly and incubate cells for 2 min. at 37°C.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells with centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 2.0 E+06 cells per mL with fresh freezing medium.
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

4.8 Preparing Cells for Experiments

- Transfect cells with cDNA encoding for any NR2 subunit
  (Attractene, Qiagen or Lipofectamine, Life Technologies)
- 6 h after transfection, add 1 µg/mL Tetracycline and 100 µM APV [(2R)-amino-5-phosphonovaleric acid, NMDA receptor blocker] (final concentration) to medium.
  Tetracycline induces expression of NR1, while APV blocks functional NMDA receptors to prevent cell death
- NMDA blocker APV should be removed shortly prior to experimental start by washing 3 times with medium or control solution.
- Cells can be used 24-72 h after induction

5 CONTACT INFORMATION

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