Data Sheet

HEK293 GABAA Receptor α1β2γ2 (short form) Cell Line

SCC460

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

Background

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system. It acts through GABA-A and GABA-B receptors. GABAA receptors are widespread in brain.[1] They are ligand gated chloride ion channels and play a major role in modulating fast inhibitory neurotransmission.[2] Dysfunction or mutation of this receptor results in neurological disorders including epilepsy and schizophrenia.[3] They are the targets for various drugs including sedatives, hypnotics, anxiolytics, anticonvulsants as well as other general anesthetics.[4] Structurally GABAA receptors (GABAA Rs) are heteropentamers. Receptors with two α subunits, two β subunits and one γ subunit is most observed with the prevalent native subunit combinations of α1 and β2 or α5 and β3 with γ2 subunit.

The γ2 subunit contributes to postsynaptic clustering of GABAA Rs during synaptogenesis, postsynaptic localization and maintenance of GABAA R at mature synapses. Alternative RNA splicing results in the expression of two isoforms of the γ subunit (γ2S, short isoform; γ2L, long isoform). γ2S is expressed early in development, while γ2L increases markedly in the adult brain. The long isoform, γ2L, has insertion of eight amino acids and regulates synaptic stabilization and trafficking. GABAA Rs containing γ2S are less stable within the synapse and migrate to extra-synaptic compartments. These receptors exhibit enhanced GABA transmission and reduced desensitization compared with γ2L-containing GABAA Rs.[5] Pharmacologic sensitivity and physiologic characteristics of these receptors are determined by these constituent subunits.

The receptors with short splice variant of γ2 subunit are insensitive to volatile anesthetic, isofluorane.[6] The HEK293 GABAA Receptor α1β2γ2 (short form) Cell Line, SCC460, presented herewith, are transfected to stably express α1β2γ2 (short isoform) of GABAA receptor. They can be utilized to efficiently study their function and pharmacologic effects of various compounds *in vitro*.

Source

HEK293 GABAA Receptor α1β2γ2 (short form) Cell Line was derived from gene edited HEK293 cells.[7]

Short Tandem Repeat

D3S1358:	15,16,17	D13S317:	12
D7S820:	11	D16S539:	9, 13
vWA:	15,16,17,18,19	TH01:	7, 9.3
FGA:	22, 23	TPOX:	11,12
D8S1179:	11,12,13,14,15,16	CSF1PO:	11,12,13
D21S11:	28,29.2	Amelogenin:	X
D18S51:	17,18,19	Penta D:	9,10
D5S818:	8,9	Penta E:	7, 15

Cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

HEK293 GABAA Receptor α1β2γ2 (short form) cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.

Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.

Cells tested negative for mycoplasma.

Storage and Handling

HEK293 GABAA Receptor α1β2γ2 (short form) cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Materials Required (Not provided)

- DMEM, high glucose (D6429)
- Fetal bovine serum (ES-009-B)
- Glutamine (TMS-002-C)
- Penicillin/streptomycin (P4333)
- Puromycin (P7255)
- Accutase® (SCR005)
- D-PBS without Calcium & Magnesium (D8537)

Representative Data

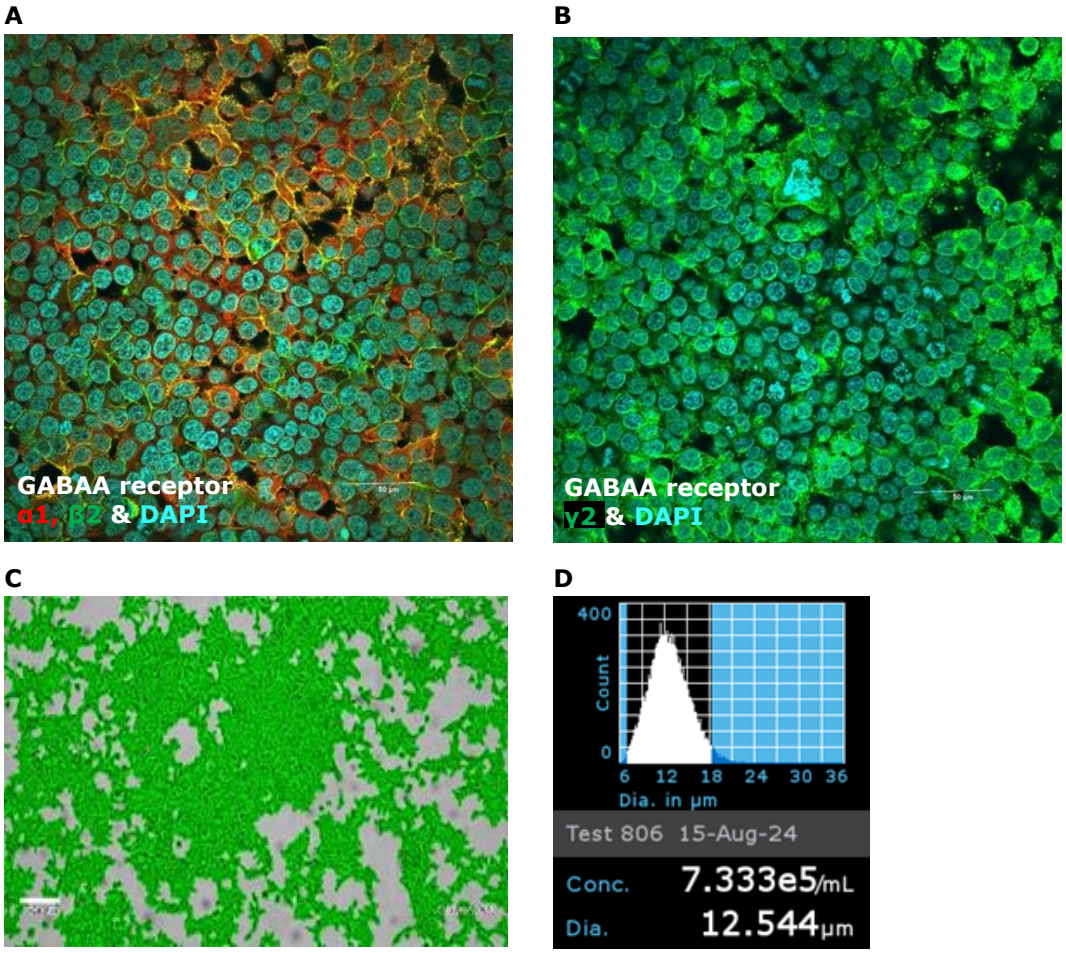

Figure: 40X Immuno-fluorescence images of SCC460 cells showing expression of GABAA Receptor subunits-α1 (abcam, ab281915) and β2 (MAB341, **A**) and γ2 (MABN875, **B**)**.** (**C**) Masked 10X phase contrast Millicell® DCI image of SCC460 on Day 3 showing 68% confluency. (**D**) Graph showing SCC460 cell count measured by Scepter™ 3.

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.
2. Cells are thawed and expanded in HEK293 GABAA Receptor α1β2γ2 (short form) cell Expansion Medium comprising of DMEM-High Glucose medium containing 10% FBS, 2 mM L-Glutamine, Pen/Strep and 2 µg/mL Puromycin.
3. Remove the vial of frozen HEK293 GABAA Receptor α1β2γ2 (short form) cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. Do not vortex the cells.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. In a laminar flow hood, use a 1-2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

6. Using a 10 mL pipette, slowly add dropwise 6 mL Expansion Medium (Step 2 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
7. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.
8. Do not vortex the cells.
9. Centrifuge the tube at 300 *x g* for 5-8 minutes to pellet the cells.
10. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
11. Resuspend the cells in 15 mL of expansion Medium.
12. Transfer the cell mixture to a T75 tissue culture flask.
13. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. HEK293 GABAA Receptor α1β2γ2 (short form) cells can be passaged at ~85% to 90 confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of HEK293 GABAA Receptor α1β2γ2 (short form) cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® and incubate in a 37 °C incubator for 3 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 8 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration. Do not vortex the cells.
11. Count the number of cells and plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

1. HEK293 GABAA Receptor α1β2γ2 (short form) cells may be frozen in expansion Medium (without Puromycin and without Pen/Strep) supplemented with 10% DMSO.
2. The cells can be stored at −80 °C overnight in a Nalgene® slow freeze Mr. Frosty® container and can subsequently be transferred and stored in liquid nitrogen.

References

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