Data Sheet

HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) Human Cell Line

SCC461

Pack Size: ≥ 1 x 10^6 viable cells

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. $GABA_A$ receptors are widespread in the brain occurring principally in the synapses.[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 and mental illnesses including epilepsy[3] and schizophrenia.[4] $GABA_A$ receptors are the targets for various drugs including sedatives, hypnotics, anxiolytics, anticonvulsants as well as other general anesthetics.[5] Structurally $GABA_A$ receptors are heteropentamers. So far, 19 subunits (α1–6, β1–3, γ1–3, Δ, ε, π, θ, ρ1–3) have been identified in the mammalian brain.[6] Receptors with two α subunits, two β subunits and one γ subunit are most observed with the prevalent native subunit combinations of α_1 and β_2 or α_5 and β_3 with γ_2 subunits. Additionally, alternative splicing of subtypes leads to further diversity into various isoforms. Pharmacologic sensitivity and physiologic characteristics of these receptors are determined by these constituent subunits. The receptors with short splice variant of γ_2 subunit are insensitive to the volatile anesthetic isofluorane.[7] Characterizing the pharmacologic effectof these specific receptor subtypes is of prime importance clinically. HEK293 cells are transfected to stably express $\alpha_1\beta_2\gamma_2$ (long isoform) of the $GABA_A$ receptor and can be utilized to efficiently study their function and pharmacological effects ofvarious compounds *in vitro*.

Source

The HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell line was derived from gene-edited HEK293 cells.[8] The parental HEK293 cells were transfected with sheared adenovirus DNA.[9]

Short Tandem Repeat

D3S1358:	15, 16, 18	D18S51:	17, 18	CSF1PO:	11, 12
D7S820:	10, 11, 11.1	D5S818:	8, 9	Amelogenin:	X
vWA:	16, 18, 19, 20	D13S317:	11, 12, 14, 15	Penta D:	9, 10, 11
FGA:	23	D16S539:	9, 12, 13	Penta E:	7, 15
D8S1179:	11, 13, 14	TH01:	7, 9.3		
D21S11:	28, 29, 30.2, 31.2	TPOX:	11, 12		

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

- Each vial contains ≥ 1 x 10^6 viable cells
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long 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.

Representative Data

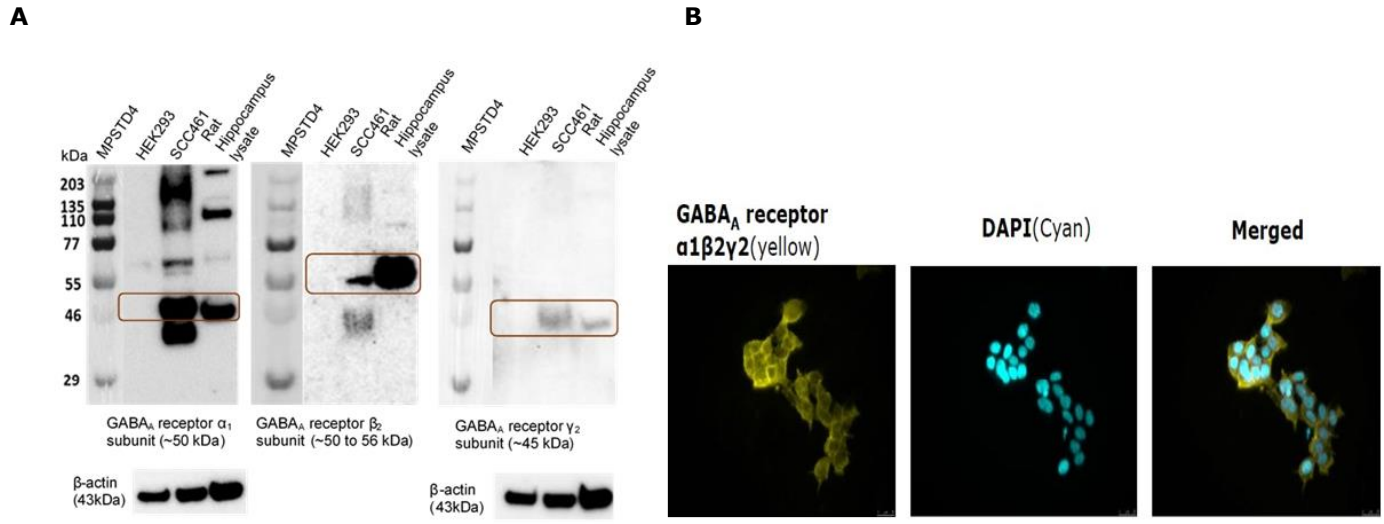

Figure 1. (**A**) Western Blot analysis of HEK293 (negative control), HEK293 GABA$_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell line (SCC461) and rat brain hippocampal (positive control) lysates using our antibodies for various GABA$_A$ receptor subunits (06-868 for α_1 subunit, AB5561 for β_2 subunit and MABN875 for γ_2 subunit) and β- actin (A3854) respectively. (**B**) Immunofluorescence images of SCC461 cells showing expression of GABA$_A$ Receptor $\alpha_1\beta_2\gamma_2$ protein (Abcam ab281915).

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 GABA$_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell Expansion medium comprising of DMEM-High Glucose medium (D6429) containing 10% FBS (for example, ES-009-B), 2 mM L-Glutamine (TMS-002-C), Pen/Strep (P4333) and 2 µg/mL Puromycin (P7255).
3. Remove the vial of frozen HEK293 GABA$_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long 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.

   Important: 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- or 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 HEK293 GABA$_A$ Receptor $\alpha_1\beta_2\gamma_2$ Expansion Medium (Step 1above) 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.

   Important: Do not vortex the cells.
8. Centrifuge the tube at 300 x *g* for 5-8 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).

10. Resuspend the cells in 15 mL of HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$(long form) cell Expansion Medium.
11. Transfer the cell mixture to a T75 tissue culture flask.
12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long 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 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long 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 HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell 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 HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    Important: Do not vortex the cells.
11. Count the number of cells.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cells may be frozen in HEK293 $GABA_A$ Receptor $\alpha_1\beta_2\gamma_2$ (long form) cell expansion medium (without Puromycin and Pen/Strep) supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

Important: Ensure the freezing medium has NO Puromycin and NO Pen/Strep.

References

1. Br J Psychiatry 2001; 179: 390-396. PMID: 11689393.
2. Neuron 1994; 12(1): 61-71. PMID: 8292360.
3. Brain Res 2019; 1714: 234-247. PMID: 30851244.
4. Nat Rev Neurosci 2008; 9(5): 331-343. PMID: 18382465.
5. Curr Opin Struct Biol 2019; 54: 189-197. PMID: 31129381.
6. Nature 2018; 559(7712): 67-72. PMID: 29950725.
7. Anesthesiology 2004; 101(4): 924-936. PMID: 15448526.
8. Mol Pharmacol 2021; 100(1): 73-82. PMID: 33958481.
9. J Gen Virol 1977; 36(1): 59-74. PMID: 886304.

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Document Template 20306518 Ver 6.0
23027120 Ver 1.0, Rev 17JAN2024, DP, AB