REF: P30109

INNOPROT STABLE RECOMBINANT GPCR CELL LINES

HUMAN BRADYKININ RECEPTOR B_1 STABLE CELL LINE

Product Name:	$BDKRB_1$/HEK293
Also Known As:	$BKRB_1$
DNA Accesion Number:	GenBank NM_000710
Host Cell:	HEK293
Format:	2 cryopreserved vials (clone “2” & clone “7”)
Quantity:	> 3 x 10^6 cells / vial
Storage:	Liquid Nitrogen

Background

Bradykinin receptor B₁. BDKRB1 gene encodes a protein that is one of two Bradykinin receptors. The **bradykinin receptor** family is a group of G-protein coupled receptors whose principal ligand is the protein bradykinin. This gene encodes a receptor for bradykinin B1. Bradykinin, a 9 aa peptide, is generated in pathophysiologic conditions such as inflammation, trauma, burns, shock, and allergy. The protein encoded by this gene is one of these receptors and is synthesized de novo following tissue injury. Receptor binding leads to an increase in the cytosolic calcium ion concentration, ultimately resulting in chronic and acute inflammatory responses.

Material Provided

Innoprot provides two vials of stably transfected cryopreserved HEK293 Cells expressing recombinant human Bradykinin receptor B_1 (GeneBank Accesion Number: NM_000710). Each vial contains > 3 x10^6 viable cells post-thawed.

Applications

- Radioligand binding assays
- Funtional assays

Quality Controls

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.

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Characterization

Our expression plasmid containing the coding sequence of human Bradykinin receptor B_1 (BDKRB1) was transfected in HEK293 cells, using calcium phosphate method. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

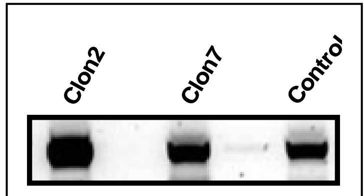
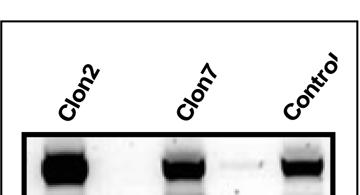

Fig.1. Clones BDKRB1 mRNA expression.

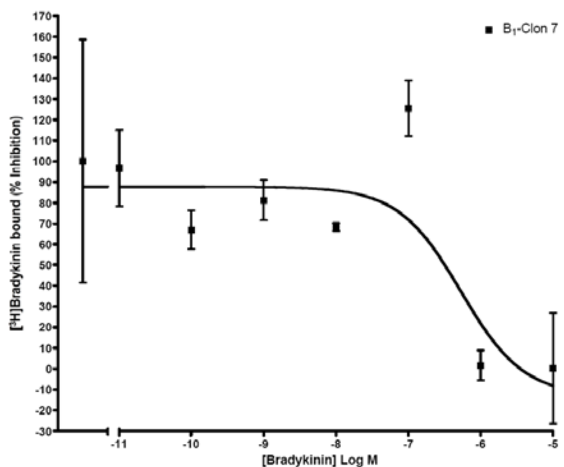

Fig.3. Inhibition percentage assay curve

Binding assay

Clon 2 or 7 (35 ug protein/well) was assayed with **[^{3}H] bradykinin** (1 nM) and increasing concentrations of no radioactive competitor.

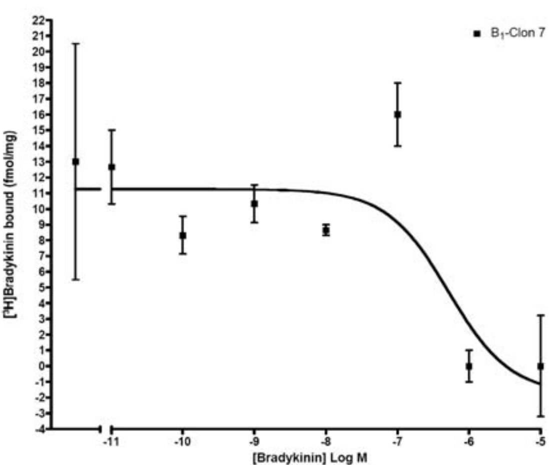

Fig.4. Receptor membrane density assay curve.

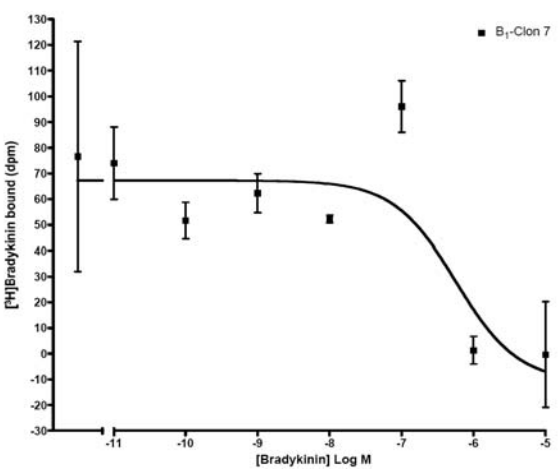

Fig.2. Competition binding assay curve

Data	IC_{50}	B_{max}	pKi
Clone 2	617 nM	47316 fmol/mg	6,9
Clone7	566 nM	5801 fmol/mg	7

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INSTRUCTIONS FOR CULTURING CELLS

A. Complete Growth medium

1. DMEM; 10% FBS, 200 ug/ml Gentamicin, 250 µg/ml, G418 (receptor expression selection).

B. Set up culture after receiving

1. Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml). Leave the flask in incubator overnight (minimum one hour at 37ºC incubator).

2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

4. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Centrifuge at 125xg for 5 min to collect the cells Remove the cap, being careful not to touch the interior threads with fingers . Remove the frozeen media using 1 ml eppendorf pipette and gently resuspend the contents of the vial in 1ml of pre-warmed complete growth media.

5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels containing 9ml pre-warmed complete growth media. A seeding density of 3.10^6 cells/cm^2 is recommended.

6. Place the culture vessels to the incubator.

7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be double after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.

2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.

   2. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

   3.

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Subculture:

1. Subculture the cells when they are over 90% confluent.

2. Prepare poly-L-lysine coated cell culture flasks.

3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37ºC waterbath prior to use.

4. Rinse the cells with DPBS.

5. Incubate cells with 5 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 5ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.

8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.