B2R- **Bradykinin receptor 2**

Product specification

Acronym: B2R
Class: Receptor
Origin: Human
Molecular weight: 44 kDa
Application: Screening & display technologies, Structural biology, Antibody development

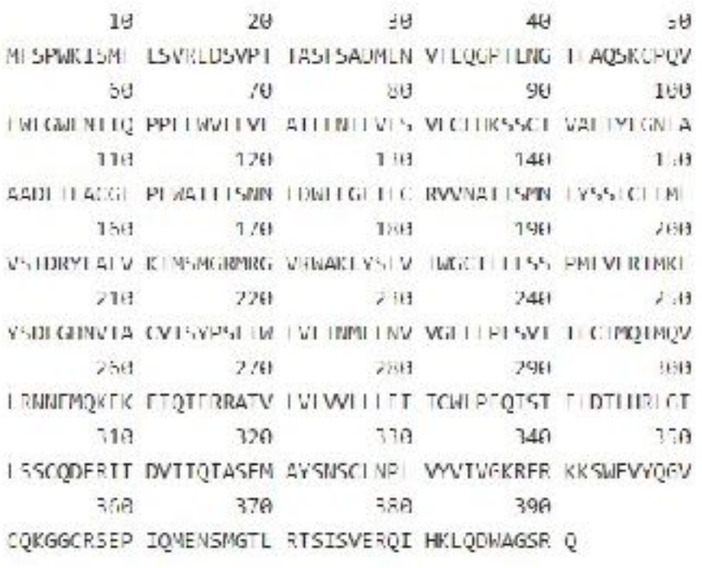
Purity: >60%
Activity: Proven by SPRi
Length: Full Length
TMD: 7
Biological function: Slow contraction of various smooth muscles

Product description

The B2R is a receptor for bradykinin. It is associated with G proteins that activate a phosphatidylinositol-calcium second messenger system. The 9 aa bradykinin peptide elicits many responses including vasodilation, edema, smooth muscle spasm and pain fiber stimulation. The B2 receptor mediates slow contraction of various smooth muscles including veins, intestine, uterus, trachea and lung, inducing endothelium-dependent relaxation of arteries and arterioles, and stimulates natriuresis/diuresis in kidney. The B2 receptor couples to Gq and Gi, Gq stimulates phospholipase C to increase intracellular free calcium and Gi inhibits adenylate cyclase. Furthermore, the receptor stimulates the mitogen-activated protein kinase pathways. The B2 receptor also forms a complex with angiotensin converting enzyme (ACE) and this is thought to play a role in cross-talk between the renin-angiotensin system (RAS) and the kinin-kallikrein system (KKS).

Protein Source: hB2R wild type protein (Human B2R):

Fig.1: AA sequence of hB2R protein



Affinity Tag: Histidine tag fused to the N-terminal end of the protein.

Production conditions: hB2R is expressed in a cell-free expression system in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week.

Quality analysis

Purity: Typically > 60% as determined by SDS-Page and Coomassie Blue staining.

Purification procedure: As standard, hB2R proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

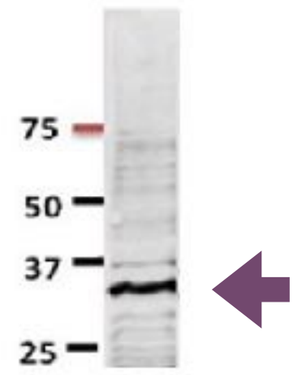


Fig.2: Proteoliposome hB2R after purification (Western blot identification).

Assessment of functionality

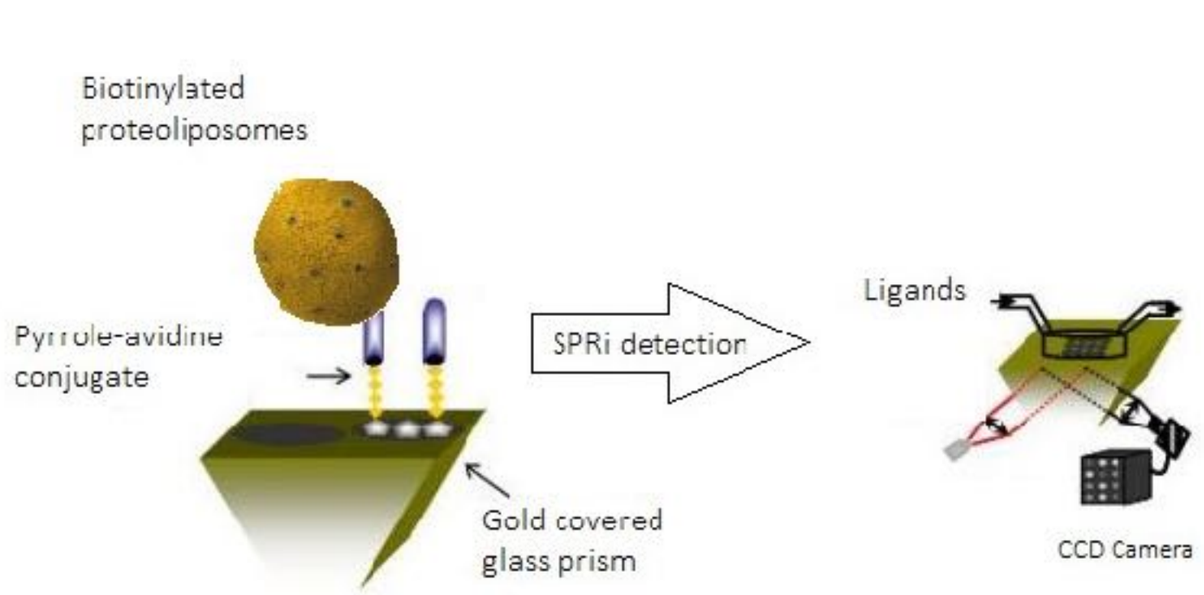
Methods: SPRi Detection

The binding properties of B2R proteoliposomes have been validated using Horiba Scientific SPRi platform (SPRi-Plex II). Small molecule and peptide ligands were injected on a biochip grafted with B2R biotinylated proteoliposomes. We detected specific interactions between B2R proteoliposomes and different ligands. The signal was dose dependent. No signal was observed on the negative control spots (proteoliposomes with non-relevant membrane protein). B2R and negative control were captured on biochip. Ligands (HOE140, Bradykinin, and specific antibody) were injected over the captured receptors at different concentrations (between 1nM and 40µM) at a flow rate of 50 µL/min. Association was monitored for 2 min and dissociation was monitored for 10 min.
All compounds were diluted in running buffer containing D-PBS (Dulbecco's Phosphate Buffered Saline without Calcium and Magnesium), 50mM MgCl2.
The binding responses of ligands were normalized to the density of proteoliposomes on the surface.
Data were analyzed using SPRi1000 software.

Immobilization by electro-polymerization on gold surface

Avidin was immobilized on a gold-coated glass prism by electrochemical copolymerization of pyrrole-avidin conjugates. Electrospotting is carried out on the gold surface with needle containing the solution to be copolymerized. Biotinylated B2R proteoliposomes and biotinylated proteoliposomes with non-relevant membrane protein (negative control) were deposited on pyrrole-avidin spots (without electro-polymerization). Each proteoliposome condition was spotted in five copies using an Omnigrid robotic arrayer (HORIBA Scientific); 40 spots (400µm diameter) were deposited on the surface of the biochip (Figure 3).

Fig.3: Schematic of proteoliposome immobilization and ligand capture method. Avidin pyrroled was immobilized on the gold surface, proteoliposomes were deposited on the avidin spots and the ligands were injected on all spots.



The capture of the ligands was performed at 20°C using SPRi-Plex II after calibration with a known analyte concentration and refractive index. A reflectivity for each spot is measured and a corrective factor is applied.

2-Results

A. HOE binding to B2R proteoliposomes

The HOE140 peptide is a selective B2 bradykinin receptor antagonist. To validate the functional integrity of the B2R proteoliposomes, the binding of HOE140 to the B2R proteoliposomes was tested. In the present experiment, solution of HOE140 was diluted in running buffer and injected in a concentration range between 1nM and 40µM.
In this present experiment, the kinetic analysis was based on single-cycle kinetics. The HOE140 peptide is injected sequentially in the same cycle with no regeneration between sample injections. This approach requires less times for a complete analysis and above all allows analysis without regeneration, step that can damage the proteoliposomes. Sensorgrams of the analyte interactions were recorded (Figure 4). These kinetic curves corresponded to an average of 5 spots. This response was specific; a slight binding was detected for the negative control.

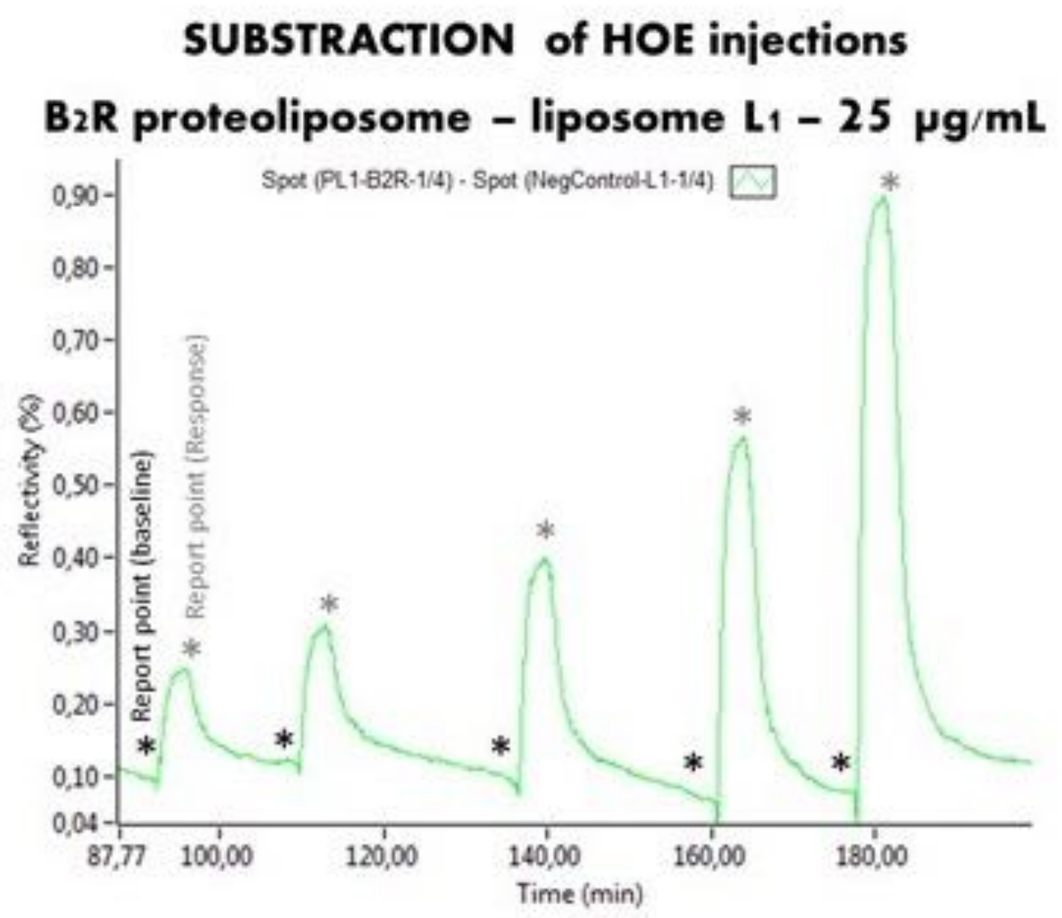


Fig.4: Single-Cycle Kinectics: sensorgrams obtained after HOE140 injections and subtraction of SPR signal obtained on the negative control spots. HOE140 peptide was injected successively at different concentrations

As it can be seen in Figure 4, the behavior of binding was specific and concentration dependent. The binding of HOE140 to negative control spots stayed slight and the specific binding was significant.

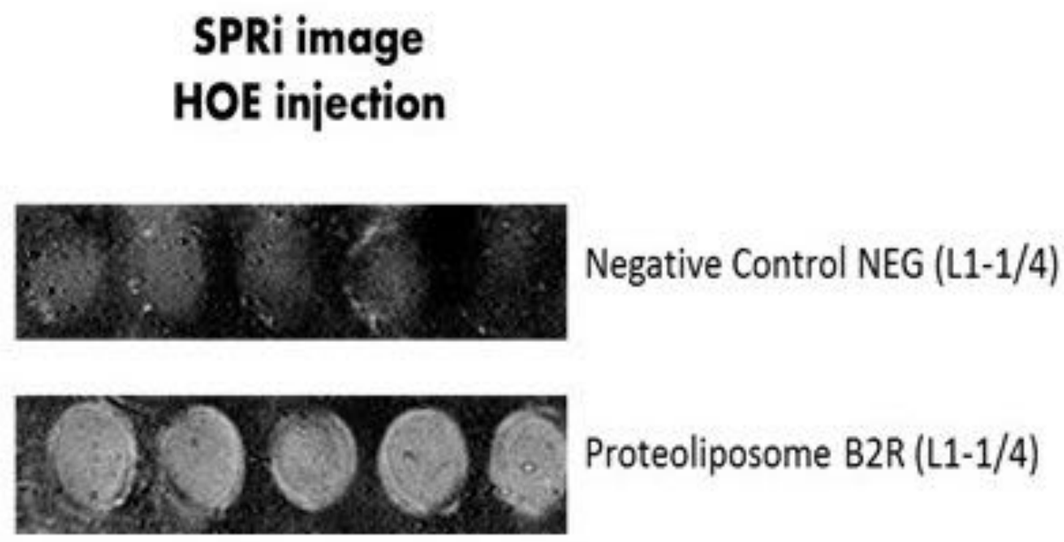


Fig.5: SPR image of the surface functionalized by negative control proteoliposome or B2R proteoliposome (five identical spots).

In the Figure 5, we can see the SPR image obtained after injection of HOE140 at 10µM. No signal was visible on negative spots. However, HOE140 binding to the B2R spots were observed.

Formulation

Buffer: Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage. Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 10µg, 20µg ,100 µg , 200 µg, 500 µg,bulk

Need a specific amount, a quote or any additional information? Contact-us

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