B2R- Bradykinin receptor 2 # PL080

Product specification

Acronym: B2R
Synonyms: BDKRB2, BKR2
Origin species : Human
Protein reference : P30411 (UniProtKB)
NP_000614 (GenBank)
Family: Receptor

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Gln391
Tag : 6xHis tag (N-ter)
Cleavage site: Factor Xa
Product MW: 47.0 kDa
Application: Drug screening & discovery, antibody development, structural biology

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).

Recombinant protein sequence

His tag – factor X cleavage site –
MFSPWKISMFLSVREDSVPTTASFSADMLNVTLQGPTLNGTFAQSKCPQVEWLGWLNTIQPPFLWVLFVLATLENIFVLSVFCLH
KSSCTVAEIYLGNLAAADLILACGLPFWAITISNNFDWLFGETLCRVVNAIISMNLYSSICFLMLVSIDRYLALVKTMSMGRMRGVR
WAKLYSLVIWGCTLLLSSPMLVFRTMKEYSDEGHNVTACVISYPSLIWEVFTNMLLNVVGFLLPLSVITFCTMQIMQVLRNNEMQ
KFKEIQTERRATVLVLVVLLLFIICWLPFQISTFLDTLHRLGILSSCQDERIIDVITQIASFMAYSNSCLNPLVYVIVGKRFRKKSWEVY
QGVCQKGGCRSEPIQMENSMGTLRTSISVERQIHKLQDWAGSRQ

Quality analysis

Purity:
Liposomes are directly incorporated into the Cell-Free reaction, thus, some impurities from the *E.coli* lysate might be present in the proteoliposomes.
A negative control (proteoliposomes without the protein of interest) can be provided (useful for screening, immunization…).
The purity can be improved by protein expression in detergent and relipidation after purification step(s).

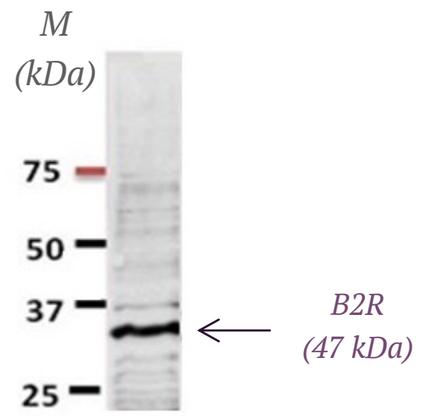

Fig.1: *Identification of B2R in the proteoliposomes by Western Blot (using an anti-6xHis antibody).*

Purification procedure: B2R proteoliposomes are purified on a sucrose gradient.

NB : Migration of membrane proteins on SDS-PAGE can result in « gel shifting » due to the presence of hairpins (helix-loop-helix)[1-3].

References :
1 – Rath A., et al., Detergent binding explains anomalous SD-PAGE migration of membrane proteins PNAS, 2009 Feb 10, vol. 106
2 – Rath A., et al., Acrylamide concentration determines the direction and magnitude of helical membrane protein gel shifts, PNAS, 2013 Sep 24, 110(39)
3 – Rath A., et al., Correction factors for membrane protein molecular weight readouts on sodium dodecyl sulfate-polyacrilamide gel electrophoresis, Anal. Biochem., 2013 Mar 1, 434(1)

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 1 nM and 40 µM) 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 (400 µm diameter) were deposited on the surface of the biochip (Figure 2).

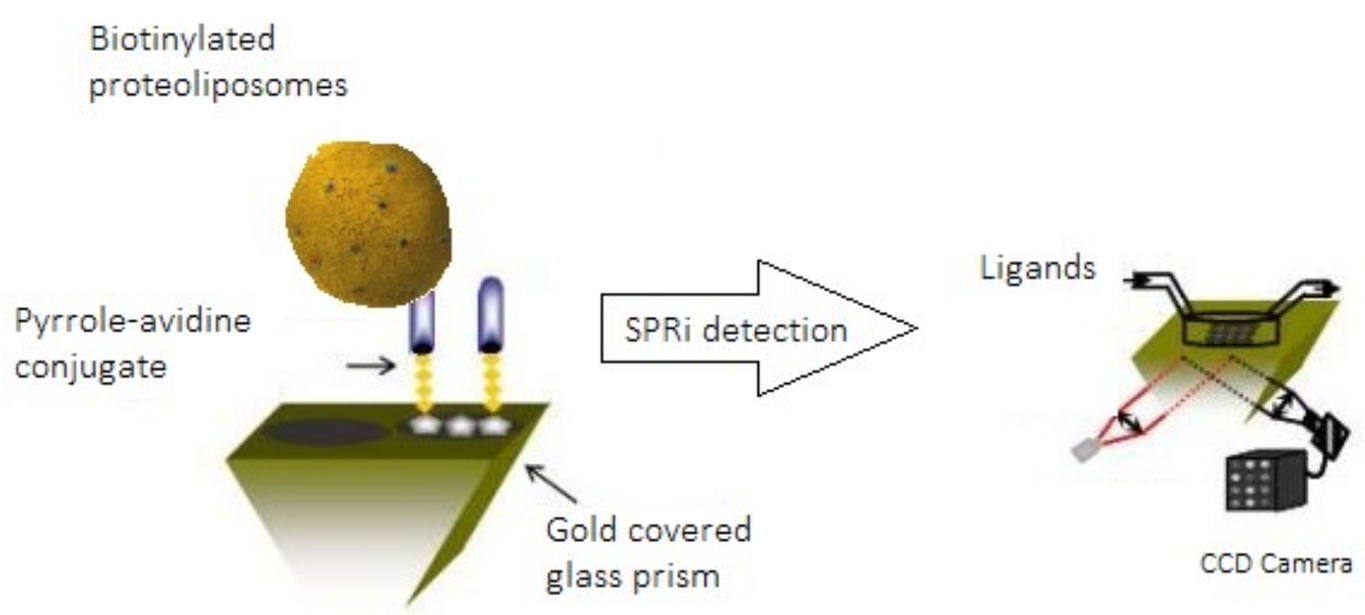

Fig.2*: Schematic of proteoliposome immobilization and ligand capture method. Avidin pyrroled was immobilized on the gold surface, proteoliposomes were deposited on the avidin spots and the ligands were injected on all spots.*

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 1 nM to 40 µM.
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 time for a complete analysis and allows analysis without regeneration step that can damage the proteoliposomes.
Sensorgrams of the analyte interactions were recorded (Figure 3). These kinetic curves corresponded to an average of 5 spots. This response was specific; a slight binding was detected for the negative control.

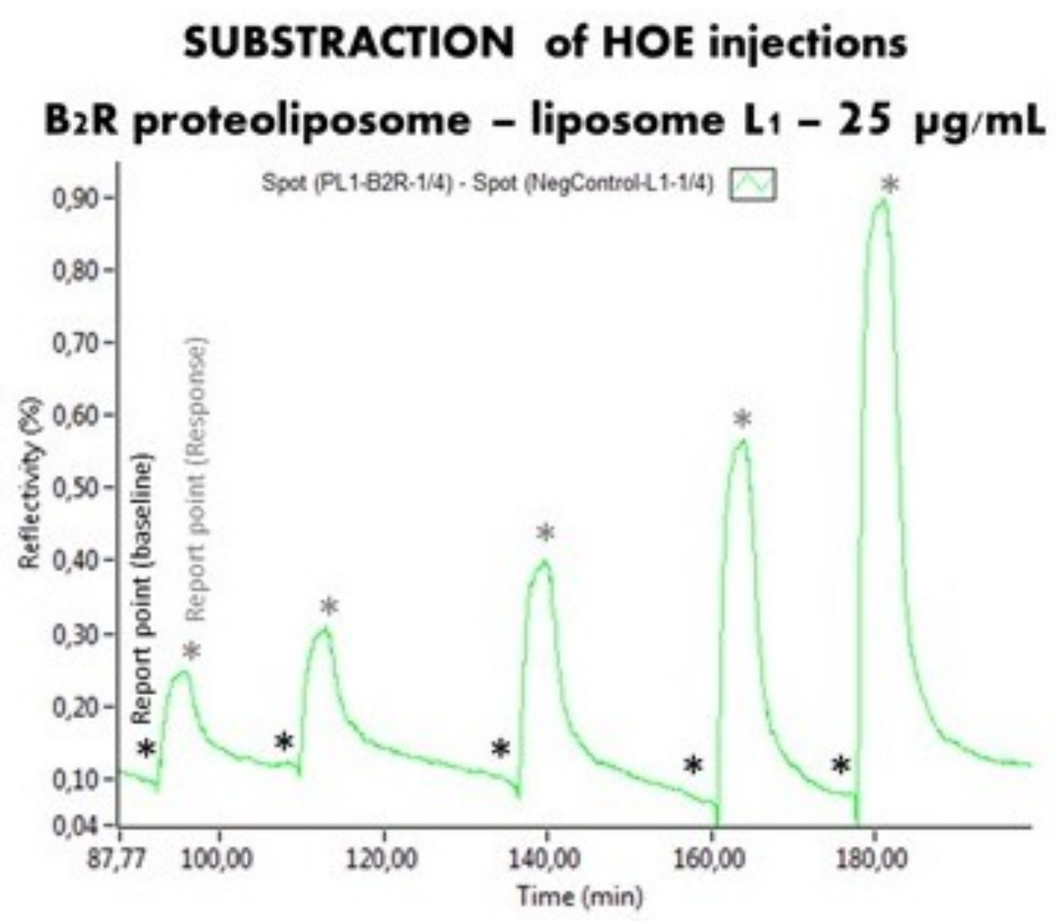

***Fig.3**: Single-Cycle Kinectics: sensorgrams obtained after HOE140 injections and subtraction of SPR signal obtained on the negative control spots. HOE140 peptide was injected successively at different concentrations.*

As it can be seen in Figure 3, 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.

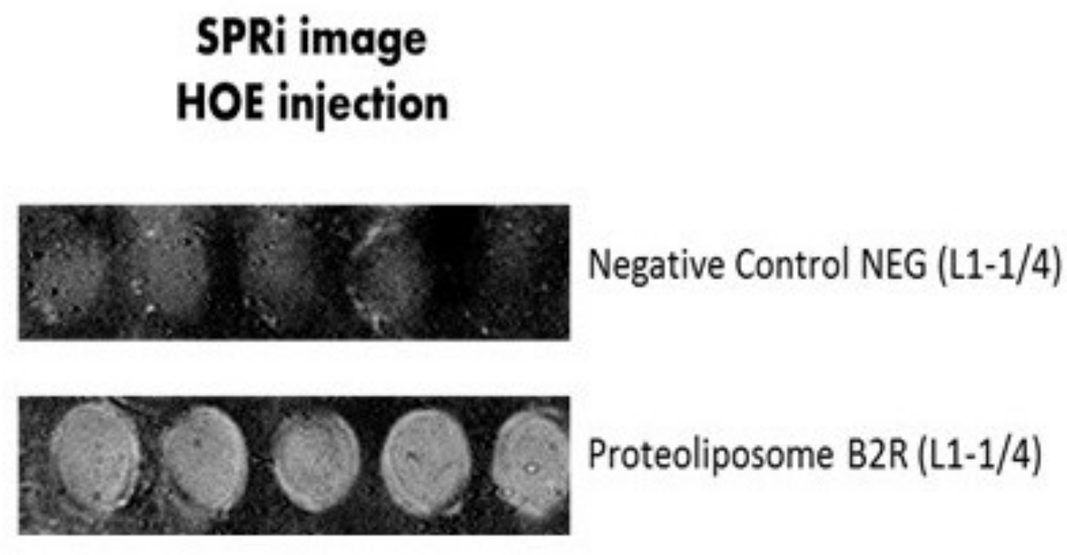

***Fig.4**: SPR image of the surface functionalized by negative control proteoliposome or B2R proteoliposome (five identical spots).*

In the Figure 4, we can see the SPR image obtained after injection of HOE140 at 10 µM. No signal was visible on negative spots and HOE140 binding to the B2R spots were observed.

Formulation

Buffer: Available in Hepes 50mM, pH 7.5, with cryoprotectants. 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: 10 µg, 50 µg ,100 µg, customized quantity on request.

Need a specific amount, a quote or any additional information?
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