# RXFP1- **Relaxin receptor 1**

## Product specification

**Acronym:** RXFP1
**Class:** GPCR Class A
**Origin:** Human
**Molecular weight:** 86,9 kDa
**Application:** Screening & display technologies, Structural biology, Antibody development

**Purity:** >70%
**Activity:** Proven by SPRi
**Length:** Full Length
**TMD:** 7
**Biological function:** hormone binding

## Product description

The relaxin/insulin-like family peptide receptor 1 is the cognate receptor for human relaxin-2 in humans. Human RXFP1 also known as LGR7 is located on chromosome 4q32.1 and is known to activate Erk1/2, tyrosine kinase(s), gene transcription, and nitric oxide (NO) signaling and can also interact with the glucocorticoid receptor (GC).
hRXFP1 is found in a wide range of reproductive tissues including ovary, uterus, placenta, mammary gland, prostate, and testis . The receptor is also present in the heart, kidney, lung, liver and blood cells as well as in a number of areas of the brain such as cortex, organum vasculosum of the lamina terminalis (OVLT), and subfornical organ (SFO). RXFP1 binds peptide hormones consequently relaxin not only has autocrine and paracrine roles but also acts as a neuropeptide. It's a potent chronotropic with inotropic effects on the human heart. Relaxin peptide (RLN) has shown therapeutic effects in an acute heart failure clinical trial

**Protein Source:** hRXFP1 wild type protein (Human RXFP1).

*Fig.1: AA sequence of hRXFP1 protein*

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

**Production conditions:** hRXFP1 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 > 70% as determined by SDS-Page and Coomassie Blue staining.

**Purification procedure:** As standard, hRXFP1 proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

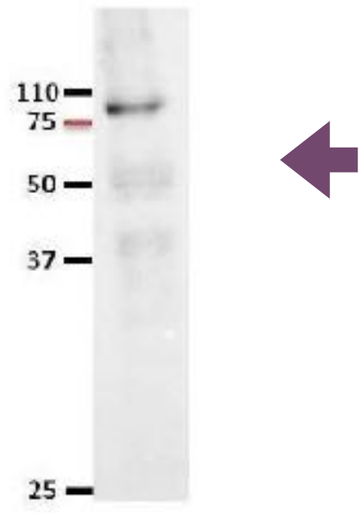



*Fig.2: Proteoliposome hRXFP1 after purification ( Western blot identification).*

After purification on a sucrose gradient, the protein appears at the right size on polyacrylamide gel.

## Assessment of the receptor functionality

### I.Introduction

The aim of this study was to develop a binding assay for hRXFP1 ligands on RXFP1 receptor proteoliposomes using SPRi. Indeed, we have developed a membrane protein biosensor assay that offers the opportunity for high-throughput label-free screening that directly measures membrane protein-ligand interactions.
A specific signal was detected between hRXFP1 receptor and its natural ligand, the relaxin. This signal is depending on the expression condition.

### II.Materials

#### 1. Relaxin ligand

Expression by cell-free and activity binding tests of hRXFP1 receptor were performed using the specifc ligand (the relaxin), soluble in the running buffer.

#### 2. Proteoliposomes

We have immobilized three different RXFP1 receptors on the biochip:
– RXFP1 receptor expressed without Calcium
– RXFP1 receptor expressed with 0,5 µM of Calcium
– RXFP1 receptor expressed with 1 mM of Calcium

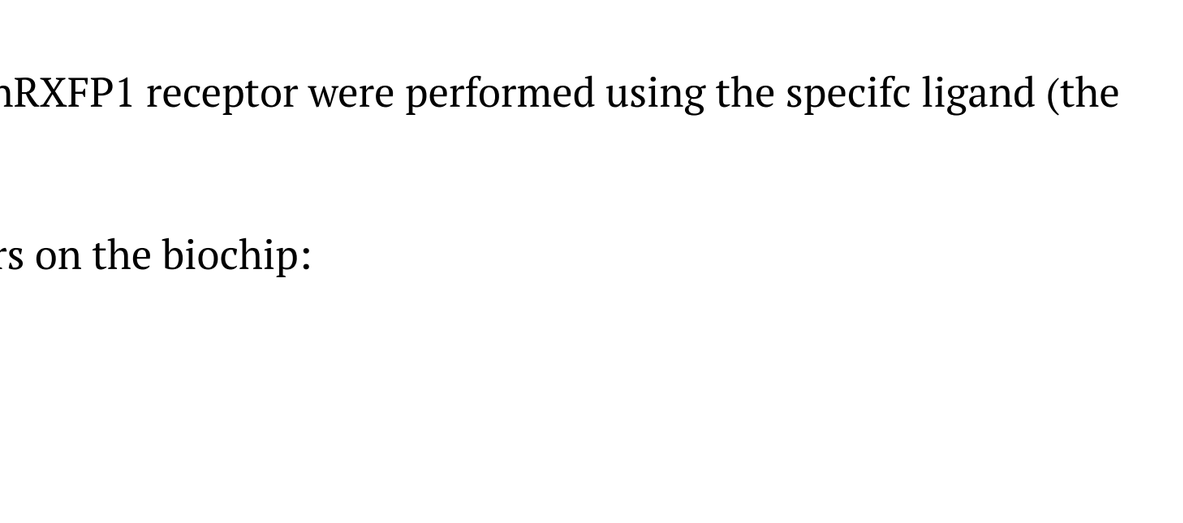
#### 3. Assay design

#### 3.1 SPRi Detection

– Immobilisation by electro-polymerization on gold surface

Avidin was immobilized on a gold-coated glass prism by electrochemical copolymerization of pyrrole-avidin conjugates. Biotinylated proteoliposomes containing hRXFP1 receptor and biotinylated proteoliposomes without this protein (negative control) were deposited on pyrrole-avidin spots. Each proteoliposome condition was spotted in five copies using an Omnigrid robotic arrayer (HORIBA Scientific) (Figure 3).

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



### 3.2 Capture of ligands on biochip

The capture of ligands was performed at 25°C using SPRiPlexII (manual instrument). The hRXFP1receptors were immobilized at 5µg/mL. The relaxin ligand was injected over the immobilized receptor at a flow rate of 50 µL/min. Association was monitored for 4 min and dissociation was monitored for 5 or 10 min.

For each experiment, all injections were carried out successively. The dissociation was completed and no regeneration was performed between ligand injections. After the injection of the compounds, the chip was rinsed with the running buffer to remove unbound compounds. All compounds were diluted in the running buffer.

The binding responses of the ligand were normalized to the density of the proteoliposomes on the surface. The data were analyzed using SPRi1000 software.

### 3.3 Experimental conditions

The ligand time injection (correlating to flow-rate) and the saturation condition were optimized in order to reduce the non-specific binding to the sensor surface (Table 1).

| | |
|---|---|
| **Running buffer** | Phosphate, MgCl2, 0.2mg/mL BSA |
| **Blocking solution** | 2 % BSA |
| **Flow rate** | 50 µL/min |
| **Kinetics angle** | 57,89° |
| **Sample loop volume** | 200µL |
| **Contact time** | 4 min |
| **Relaxin Concentration** | 5 µg/mL |
| **Report time** | Before end of sample injection |
| **Regeneration solution** | Not needed |

## III. Results

### 1.Validation of the expression :

The full length expression of RXFP1 in presence of different concentrations of calcium has been validated (Figure 4). For this, we have used the His- RXFP1 -Strep construct and we have revealed the expression with an antibody against Strep-tag. The Western blot under reducing conditions revealed the presence of a band at ≈ 70kDa corresponds to the full length of RXFP1.

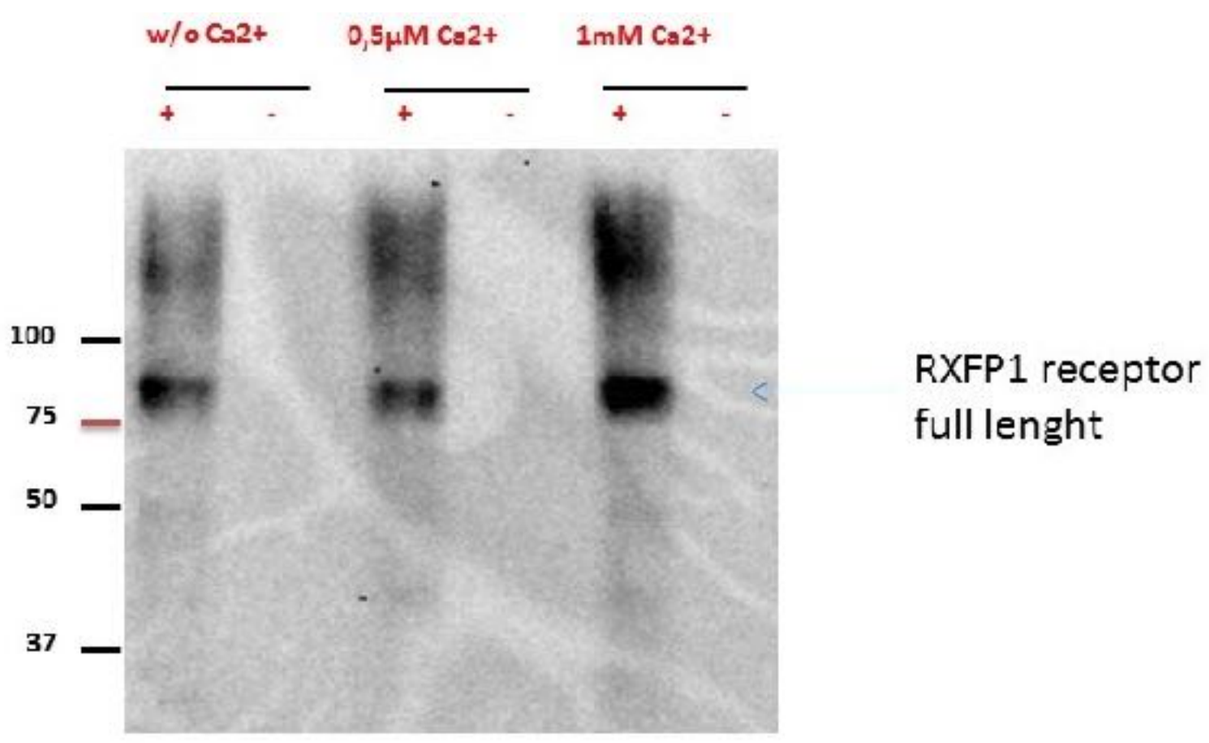



*Fig.4: RXFP1 expression in presence of different concentrations of calcium. Detection of RXFP1 receptor full lenght by Western Blot with anti-STREP antibody*

## 2.SPRi results :

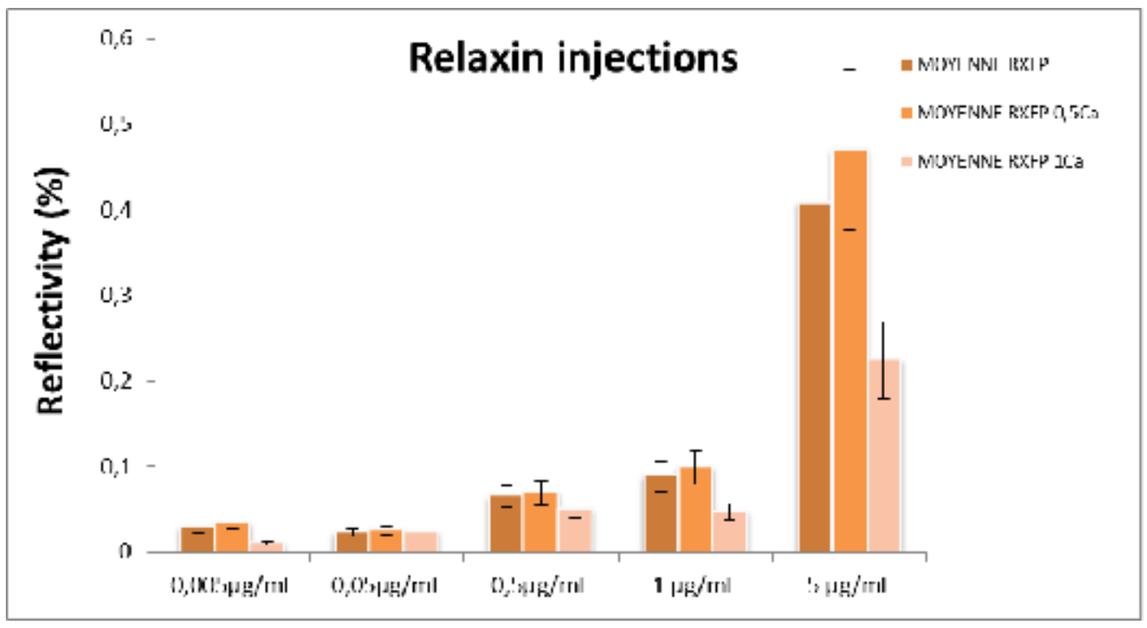
### 2.1 Relaxin ligand injection

The relaxin ligand was injected over the biochip. We have monitored the specific interaction between RXFP1 and relaxin ligand by SPRi. In this present experiment, the kinetic analysis was based on single-cycle kinetics. The relaxin ligand was injected sequentially in the same cycle.

The relaxin ligand was diluted in running buffer and injected in a concentration range between 0.5µM and 400µM. A sensorgram corresponding to a plot of response against time, showing the progress of the interaction, was recorded (Figure 3) and from sensorgram of the analyte interactions, the SPR signal (% reflectivity) at steady state (equilibrium binding) is determined and described in the Figure 3. These data correspond to the subtraction of the SPR signal on the negative control.

The compound concentration and injection time were correct since the steady state was reached and the dissociation is complete. After the injection of the compounds, the chip was rinsed with running buffer to remove unbound compounds.

*Fig.5: Validation of interaction RXFP1 / Relaxin ligand: Variations of reflectivity obtained at steady state for each sample immobilized and for each injection of relaxin ligand. These variations were calculated between two points: report point 1 was the baseline level before the injection of the compound and point 2 was the binding level at the end of the association phase (corresponding to the steady state). The reflectivity corresponds to an average of 5 spots.*




After injection of relaxin at different concentrations, we have observed a specific interaction between RXFP1 receptor and relaxin ligand. The expression condition is very critical and has a real impact to keep the receptor activity.

## 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 ,bulk