**INTRODUCTION**

Human galanin is a 30-amino acid neuropeptide that mediates a number of diverse physiological actions. Galanin exerts its action by binding to specific G-protein coupled membrane receptors. We have developed a binding assay for the detection of galanin receptor hGalR2 interaction with galanin. Assay is based on the highly sensitive fluorescence enhancement technique DELFIA®, and this format makes it possible to detect the binding of europium (Eu)-labelled ligands without filtration.

**METHODS**

The detection of receptor-ligand interaction is based on Eu-labelled human galanin (Wallac), RBhGalR2 receptor preparate (Receptor Biology, Inc.) and biotinylated WGA. These three components are incubated on 384-well streptavidin coated plate (Wallac) at room temperature for 16-19 hours. Unbound Eu-galanin is washed away and bound Eu is dissociated into the DELFIA Enhancement Solution. Eu forms highly fluorescent complexes and the fluorescence is measured using a Wallac VICTOR™ multilabel counter. (Fig.1)

**RESULTS**

![Saturation curve](image1)

**Saturation curve**

Saturation binding experiments were conducted with 0 - 16 nM Eu-galanin. The Kd-value of Eu-galanin for RBhGalR2 – receptor preparate was 2 nM (Fig. 3). Unspecific binding was determined in the presence of 1 µM unlabelled galanin.

**Displacement curve**

![Displacement curve](image2)

**Displacement of 2 nM Eu-galanin from RBhGalR2 receptor preparate by galanin and two galanin antagonists, Galanin (1-13)-Bradykinin (2-9) amide (Ki= 1.5 nM) and Galanin (1-13)-Substance P (5-11) amide (Ki= 30 nM).** (Fig. 4)

**DMSO-testing**

The effect of DMSO on the binding assay was tested using 0.75 µg of RBhGalR2. 0-2.5% had no effect on the assay, and with 5-10% of DMSO the S/N ratios were still above 11.

**CONCLUSIONS**

Our results show that this assay format is an efficient and easy way to detect receptor ligand interactions. All manipulation steps can be automated, and therefore this assay is a convenient tool for HTS laboratories. This assay principle has also been applied to other receptor binding assays.