Time-Resolved Fluorometric Receptor Ligand assay for HTS

Rita Syystö, Christel Gripenberg-Lerche, Johanna Tamminen, Jarkko Karvinen, Pertti Hurskainen, and Ilkka Hemmilä.
PerkinElmer Life Sciences, Wallac Oy, P.O.Box 10, FIN-20101 Turku, Finland

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^2™ multilabel counter. (Fig.1)

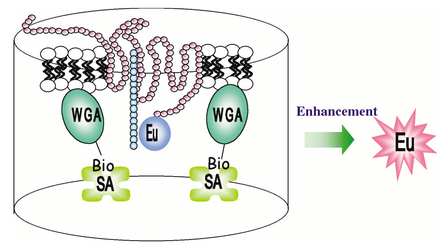

Figure 1.
The Principle of developed receptor-ligand assay

RESULTS

Membrane concentration

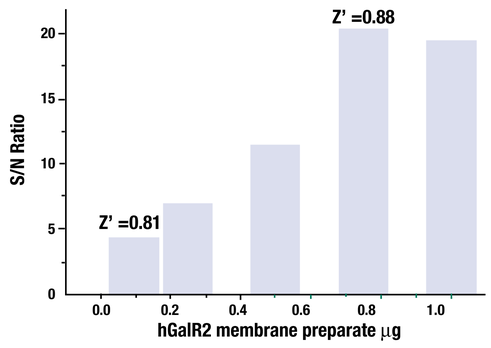

Figure 2.
Galanin assay; S/N ratios and some Z' values on 384-well plate using increasing amounts of RBhGalR2.
S/N = total signal/nonspecific binding
Z' value = 1-[(3xSDtotal+3xSDunspecific)/(total-unspecific)]

The effect of membrane concentration was measured using increasing amounts of RBhGalR2 (Fig. 2). S/N ratio 20.8 and Z' factor value 0.88 was reached with 0.75 µg of membrane. Even 0.1 µg of membrane was enough for this assay.

Saturation curve

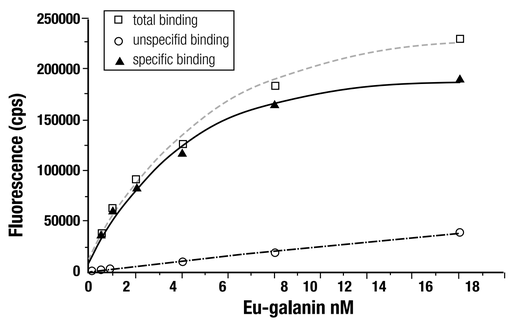

Figure 3.
The saturation binding curve of Eu-labelled galanin on 384-well plate

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

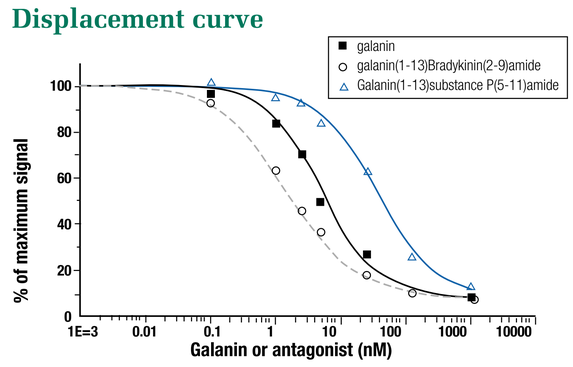

Figure 4.
Displacement of 2 nM Eu-galanin from RBhGalR2 receptor preparate by galanin and two galanin antagonists on 384-well plate

Displacement of specific Eu-galanin binding (2 nM) was conducted by unlabelled galanin (K_i= 5 nM) and two galanin receptor antagonists, Galanin (1-13)-Bradykinin (2-9) amide (K_i= 1.5 nM) and Galanin (1-13)-Substance P (5-11) amide (K_i= 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.