INTRODUCTION

The novel QRET technology is sensitive, fast, cost-effective and highly potential technique for cell-based biomolecular screening studies and diagnostics. The method relies on a single label approach and time-resolved fluorescence (TRF) detection allowing the development of entirely new types of assays in the TRF detection mode. Currently, GTP assays are based on separation using filtration and no homogeneous GTP assays are available. Here we have successfully developed for the first time a homogeneous cell membrane-based GTP assay using the QRET technique. The homogeneous assay principle is amenable to high throughput screening studies completing existing tools for cell signaling investigations. The single label approach is highly beneficial in cell-based studies because there is no need for receptor labeling. Previously, we have shown the potentiality of the method in cell-based β2-AR receptor-ligand and cAMP cell-signaling assays.

RESULTS

A homogeneous assay based on the QRET technique was developed for GTP. At agonist stimulation of α2 adrenergic receptor in CHO cell membrane and binding of Eu-labeled GTP (Wallac, Perkin Elmer Life and Analytical Sciences) to Gα protein, TR fluorescence of Eu-GTP was protected and measured at 615 nm with Victor2 multilabel counter. When no stimulation was carried out, non-bound Eu-GTP was quenched in solution. In addition, an Eu-filtration wash assay was carried out for the same α2-AR stimulation (Wallac, PE). We obtained EC50 values from 16 to 150 nM with an average CV of 13% for the QRET assay and EC50 values from 5 to 563 nM with an average CV of 8% for the Eu-filtration assay.