User Manual

Receptor Recruitment Assay KIT

For Chemiluminescent Detection of Receptor-Induced recruitment Signaling

For Drugscreen detection reagents:

Catlog Number	Unit Size
SHY-DS1250	25000 tests
SHY-DS1025	2500 tests
SHY-DS1005	500 tests

Document Number 20-1122-DS1000-R3

Overview

The Human GPCR Signaling Bioassay Kit enables a robust, highly sensitive, and easy-to-use functional cell-based assay to determine drug potency and detect neutralizing antibodies or small molecule inhibitors. The bioassay kit only contains ready-to-use cryopreserved cells needed for a complete assay, but without detection reagents, cell plating reagent, positive control agonist, or assay plates. The ready-to-use cryopreserved cells have been manufactured to ensure assay reproducibility and faster implementation from characterization to lot release. This bioassay has been optimized for a 96-well plate format. The protocol can be optimized further for running the assay in a high-throughput 384-well plate format.

Assay Principle

The Human GPCR Signaling Bioassay utilizes the Enzyme Fragment Complementation (EFC) technology to interrogate receptor activity. EFC consists of two β-galactosidase (β-gal) enzyme fragments, the Enzyme Donor (ED) and Enzyme Acceptor (EA) fragments, which independently have no β-gal activity. However, when forced to complement, they form an active β-gal enzyme that hydrolyzes a substrate to produce a chemiluminescent signal.

The Human GPCR Signaling Bioassay consists of human cells engineered to stably express an ED-tagged GPCR receptor, while EA is fused to the phosphotyrosine-binding domain of the intracellular signaling proteins, beta-arrestin. Ligand or antibody-induced activation of the receptor results in phosphorylation of the receptor's cytosolic tail. The beta-arrestin fused to EA binds the phosphorylated receptor, forcing complementation of ED and EA, resulting in formation of an active β-gal enzyme, which hydrolyzes the substrate to produce a chemiluminescent signal.

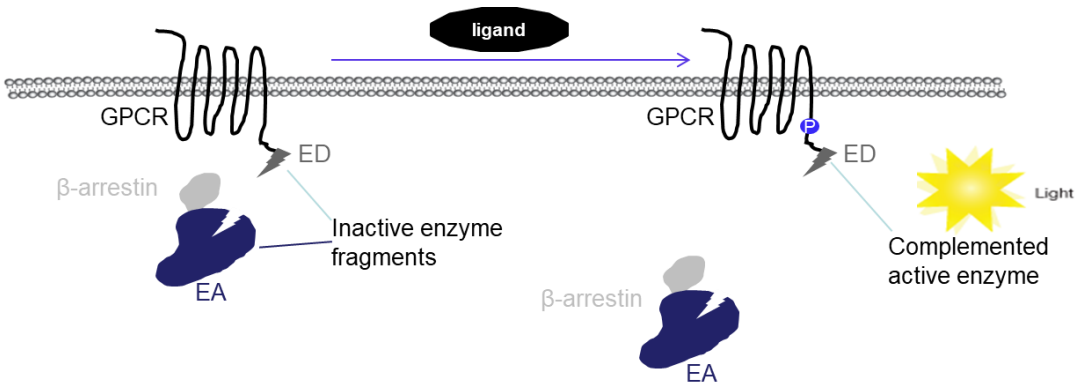

Figure 1. Assay Principle: Full-length GPCR receptor was engineered with the ED fragment fused to its C-terminus, and the beta-arrestin part was engineered with the complementary EA fragment. These constructs were stably expressed in HEK293 cells. Ligand engagement, through adding GPCR ligands, results in phosphorylation of GPCR, leading to the recruitment of beta-arrestin-EA. This forces complementation of the EFC components to create an active β-gal enzyme. This active enzyme hydrolyzes substrate to create chemiluminescence as a measure of receptor activity. Addition of an antagonist (e.g. antibody to GPCR or its ligands) blocks GPCR signaling, and will prevent complementation, resulting in a loss of signal.

Materials Provided

List of Components	Cat. Number	Size
Drugscreen detection reagents	SHY-DS1250	25000 tests
Drugscreen detection reagents	SHY-DS1025	2500 tests
Drugscreen detection reagents	SHY-DS1005	500 tests

Storage Conditions

Storage: Store at 2-8 ℃ and protect from light.

Shelf Life: Stable until expiry date (EXP) on label.

Additional Materials Required

The following equipment and additional materials are required to perform these assays:

Material	Ordering Information
Cryopreserved cells with both β-galactosidase (β-gal) enzyme fragments	**SHY-RR-XXX, please refer to Shiyuan Official Website: http://www.shybio.com.cn/**
96-Well White, flat, U or V-Bottom, Untreated, Non-Sterile Dilution Plates	Corning 3610, 3894 or 3799
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart
Sterile disposable reagent reservoir	Thermo Fisher Scientific, Cat. No. 8094 or similar
Single and multichannel micropipettes and pipette tips (10 µL-1,000 µL)	
Polypropylene tubes (50 mL and 15 mL)	
Microcentrifuge tubes (1.5 mL)	
Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (T25 and T75 flasks, etc.)	

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