Cell Meter™ Beta-Arrestin Translocation GPCR Signaling Kit

Catalog number: 36390, 36391
Unit size: 100 Tests, 200 Tests

Component	Storage	Amount (Cat No. 36390)	Amount (Cat No. 36391)
Component A: Beta-arrestin-GFP DNA	Freeze (< -15 °C), Minimize light exposure	1 vial (15 µg)	2 vials (15 µg/vial)
Component B: Transfectamine™ 5000	Freeze (< -15 °C), Minimize light exposure	1 vial (75 µL)	1 vial (150 µL)

OVERVIEW

Virtually all G protein coupled receptors (GPCRs) rapidly undergo desensitization by a common pathway upon activation by ligand binding. The binding of beta-arrestin, a cytoplasmic protein, to an activated receptor deactivates the GPCR signaling and initiates the translocation of the receptor into the cell where the ligand is removed, and the receptor is recycled back to the cell membrane. By attaching a fluorescent label, such as GFP, to beta-arrestin, the location of the receptor arrestin complex can be monitored. Since desensitization only occurs with an activated receptor, monitoring beta-arrestin translocation and subsequent receptor recycling provides a reliable method to detect the activation of a GPCR target. Cell Meter™ Beta-Arrestin translocation GPCR Signaling Kit provides a powerful functional assay to screen activities of target compounds against known or orphan GPCR targets via fluorescence imaging. The activation of the targeted GPCR induces the translocation of the fluorescence to the cell membrane and/or to endocytic vesicles.

AT A GLANCE

Protocol summary

1. Prepare cells for transfection
2. Prepare Transfectamine™ 5000-DNA mixture
3. Add Transfectamine™ 5000-DNA mixture to cell culture, and incubate overnight
4. Transfer the transfected cells to a 96-well plate 24-30 hours after transfection, and incubate the culture overnight
5. Analyze translocation induced by GPCR activation under a fluorescence microscope

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

1. Seed the cells at a density such that they will be ~60-70% confluent at the time of transfection.
2. Replace with fresh growth medium before transfection.

Note For example, replace with 2 mL of medium per well for 6-well plates and 6 mL of medium for 10 cm plates.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

beta-arrestin-GFP DNA stock solution

Add 10 µL of ddH_2O to the vial of beta-arrestin-GFP DNA (Component A), mix well to have the final concentration of 1 µg/µL.

PREPARATION OF WORKING SOLUTION

1. Mix 3 µg of DNA [for example, 1.5 µg of Beta-arrestin-GFP DNA stock solution and 1.5 µg DNA of the GPCR that you are interested] with 200 µL of serum-free medium.
2. Add 9 µL of Transfectamine™ 5000 (Component B) to the mixture.
3. Mix well and incubate at room temperature for 20 minutes.

Note The ratio of Transfectamine™ 5000 and DNA need to be optimized for different cell lines, in general, in our testings, the ratio for Transfectamine™ 5000 Transfection Reagent (µL) to DNA (µg) Ratio should be 3-5 µL : 1 µg.

Table 1. Sample rprotocols for a 6-well plate and a 10 cm plate

Component	6-well plate (per well)	10 cm plate
Fresh culture medium	2 mL	6 mL
Plasmid	3 µg	10 µg
Serum-free medium	200 µL	600 µL
Transfectamine™ 5000 Transfection Reagent	~9 µL	~30 µL

SAMPLE EXPERIMENTAL PROTOCOL

Transfection and Translocation protocol

1. Add Transfectamine™ 5000 -DNA mixture to the culture plate and incubate overnight.

Note The recombinant protein can start to be detected as early as 16 hours after transfection. The maximal expression level may be observed 72~96 hours after transfection.

2. Transfer the transfected cells to a 96-well plate 24-30 hours after transfection and incubate overnight.

3. Monitor the beta-arrestin translocation induced by the receptor activation under a fluorescence microscope with the FITC filter (Ex/Em = 488/530 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

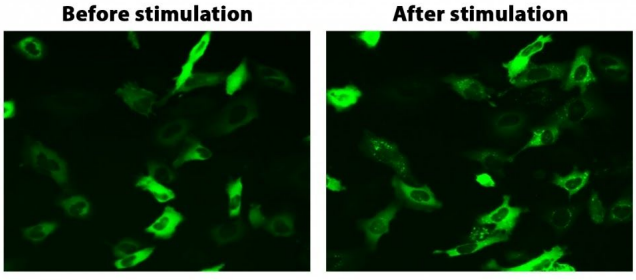

Figure 1. Translocation of beta-arrestin in HeLa cells. HeLa cells were transiently transfected with beta-arrestin-GFP and vasopressin receptor 2 (V2R). HeLa cells were cultured in a 6-well plate and grown to ~60% confluence. Equal amounts of beta-arrestin-GFP (1.5 µg) and V2R plasmids (1.5 µg) were transfected with 9 µL of Transfectamine™ 5000. Cells were transferred to a 96-well plate ~ 30 hours after transfection. Vasopressin (1 µM) was added to the cells ~ 48 hours after transfection to induce beta-arrestin-GFP translocation. Images were taken before and 2 hours after the vasopressin treatment under a fluorescent microscope using the FITC channel.

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