ALPHASCREEN APPLICATION NOTE AN005-ASC

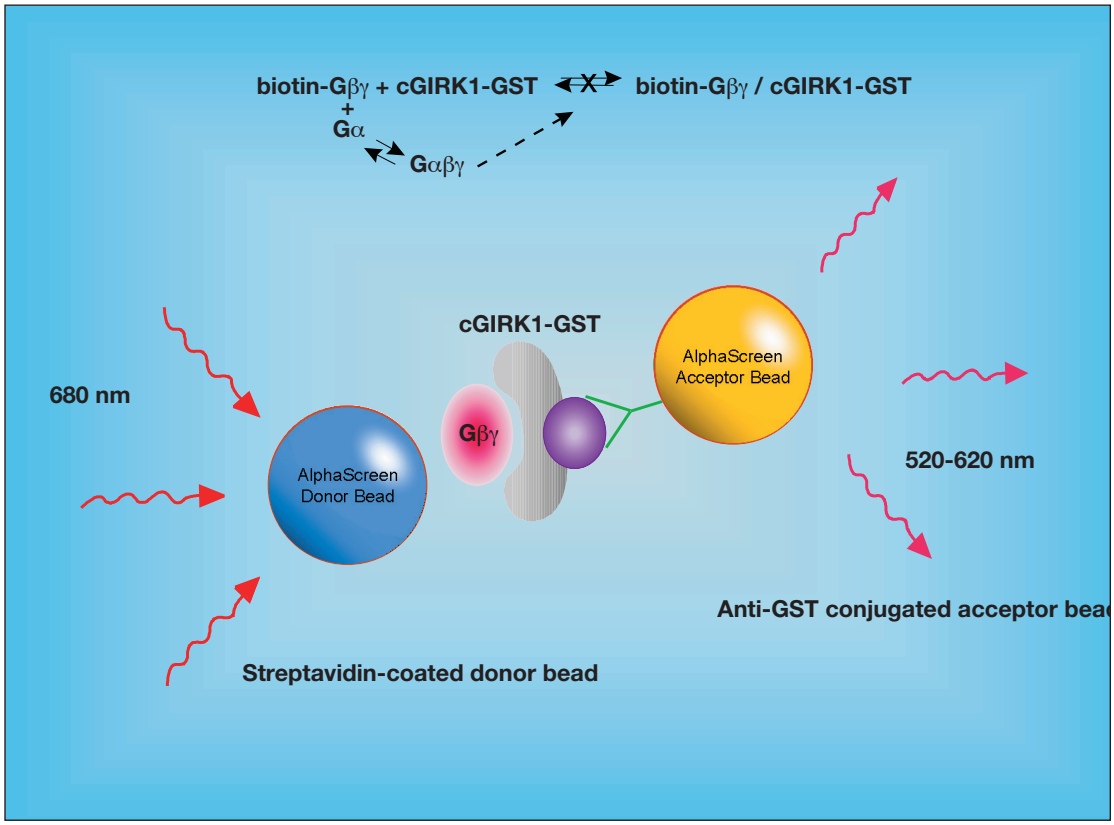
Application Note

Gβγ-GIRK1 Interaction Assay

PerkinElmer®
precisely.

Introduction

AlphaScreen™ Gßγ-GIRK1 Interaction is a fully homogeneous assay in 384-well format, designed to measure interaction between G protein ßγ subunit and the carboxyl terminal domain of the GIRK1 potassium channel (named cGIRK1). Gßγ is biotinylated and binds to streptavidin-donor beads, while cGIRK1 binds to anti GST-acceptor beads via its GST tag. Specific interaction between the two proteins leads to signal increase. The binding is fully reversible and completely abolished by competition with G protein α subunit. This assay can be easily applied to any protein-protein interaction.



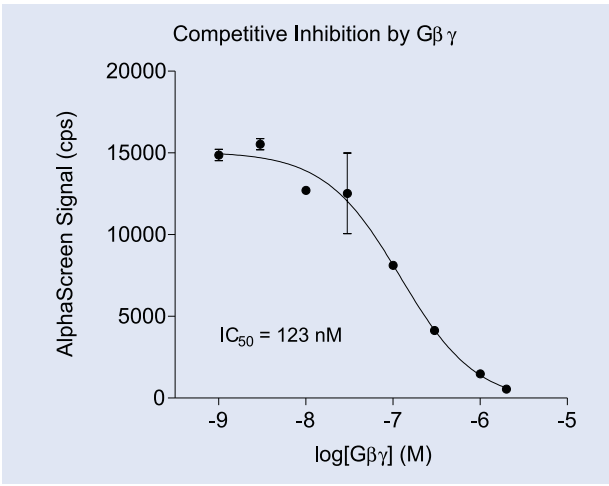
Example #1:

Competition of biotin-Gβγ binding to GST-cGIRK1 by Gβγ
Assay developed in PerkinElmer 384-well OptiPlate™

Reagents

1. Biotinylated Gβ1γ2 (12.3 µM in 20 mM Hepes, 100 mM NaCl, 0.1% CHAPS): dilute to 125 nM with assay buffer
2. GST-cGIRK1 (14.7 µM in 25 mM Hepes pH 8.0, 50 mM NaCl, 5% glycerol): dilute to 50 nM with assay buffer
3. Gβ1γ2 (22.1 µM in 20 mM Hepes, 100 mM NaCl, 0.7% CHAPS): dilute to 30 µM - 5 nM with assay buffer
4. Streptavidin-donor beads (5 mg/ml in 25 mM Hepes,pH 7.4): dilute to 50 µg/ml with assay buffer
5. Biotin-Gβ1γ2 / streptavidin-donor bead complex: mix equal volumes of biotinylated Gβ1γ2 and streptavidin-donor beads and incubate for 30 minutes at RT
6. Anti GST-acceptor beads (5 mg/ml in 25 mM Hepes, pH 7.4): dilute to 125 µg/ml with assay buffer

Assay buffer: 100 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 0.1% BSA



Protocol:

1. Add 5 µl GST-cGIRK1.
2. Add 5 µl Gβ1γ2.
3. Add 5 µl anti GST-acceptor beads.
4. Incubate 30 minutes at RT.
5. Add 10 µl biotin-Gβ1γ2 / streptavidin-donor bead complex.
6. Incubate 60 minutes at RT.
7. Read plate.

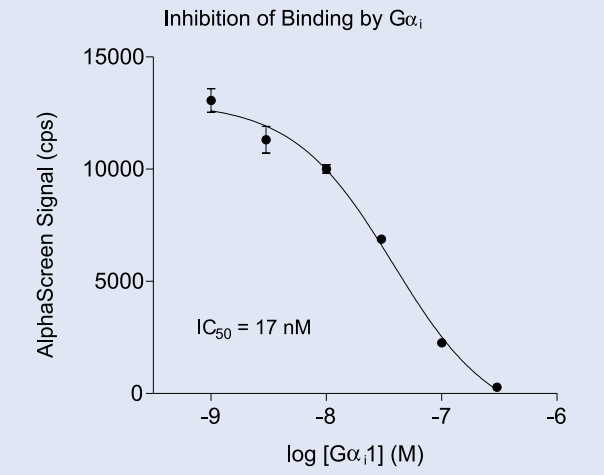
Example #2:

Inhibition of biotin-Gβγ binding to GST-cGIRK1 by $G\alpha_i$
Assay developed in PerkinElmer 384-well OptiPlate™

Reagents

1. Biotinylated Gβ1γ2 (12.3 μM in 20 mM Hepes, 100 mM NaCl, 0.1% CHAPS): dilute to 125 nM with assay buffer
2. GST-cGIRK1 (14.7 μM in 25 mM Hepes pH 8.0, 50 mM NaCl, 5% glycerol): dilute to 50 nM with assay buffer
3. $G\alpha_i1$ (11.5 μM in 20 mM Hepes, 100 mM NaCl, 0.7% CHAPS): dilute to 3 μM with assay buffer, incubate for 2h at 30°C in 25 mM Tris-HCl, pH 7.5, 0.5 mM $MgCl_2$, 100 μM GDP, then further dilute to 2.5 μM - 5 nM with assay buffer
4. Streptavidin-donor beads (5 mg/ml in 25 mM Hepes, pH 7.4): dilute to 50 μg/ml with assay buffer
5. Anti GST-acceptor beads (5 mg/ml in 25 mM Hepes, pH 7.4): dilute to 125 μg/ml with assay buffer
6. GST-cGIRK1 / anti GST-acceptor beads complex: mix equal volumes of GST-cGIRK1 and anti-GST-acceptor beads and incubate for 30 minutes at RT

Assay buffer: 100 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 0.1% BSA



Protocol:

1. Add 5 μl biotin-Gß1γ2.
2. Add 5 μl $G\alpha_i1$.
3. Add 5 μl streptavidin-donor beads.
4. Incubate 30 minutes at RT.
5. Add 10 μl GST-cGIRK1 / anti-GST-acceptor bead complex.
6. Incubate 60 minutes at RT.
7. Read plate.

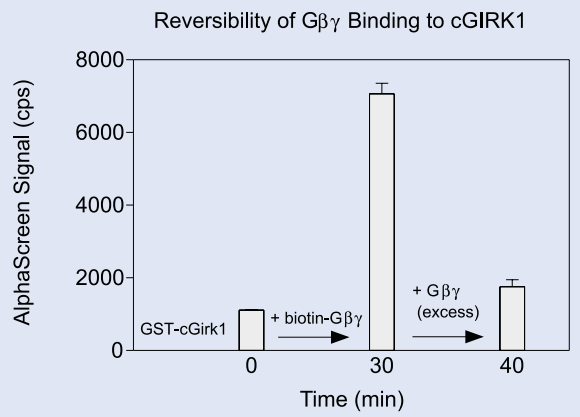
Example #3:

Reversibility of biotin-Gβγ / GST-cGIRK1 interaction
Assay developed in PerkinElmer 384-well OptiPlate™

Reagents

1. Biotinylated Gβ1γ2 (12.3 µM in 20 mM Hepes, 100 mM NaCl, 0.1% CHAPS): dilute to 125 nM with assay buffer
2. GST-cGIRK1 (14.7µM in 25 mM Hepes pH 8.0, 50 mM NaCl, 5% glycerol): dilute to 50 nM with assay buffer
3. Gβ1γ2 (22.1 µM in 20 mM Hepes, 100 mM NaCl, 0.7% CHAPS): dilute to 5 µM with assay buffer
4. Streptavidin-donor beads (5 mg/ml in 25 mM Hepes, pH 7.4): dilute to 50 µg/ml with assay buffer
5. Anti GST-acceptor beads (5 mg/ml in 25 mM Hepes, pH 7.4): dilute to 125 µg/ml with assay buffer

Assay buffer: 100 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 0.1% BSA



Protocol for binding association:

1. Add 5 µl GST-cGIRK1.
2. Add 5 µl anti GST-acceptor beads.
3. Incubate 30 minutes at RT.
4. Add 5 µl biotin-Gβ1γ2.
5. Incubate 30 minutes at RT.
6. Add 10 µl streptavidin-donor beads.
7. Incubate 10 minutes at RT.
8. Read plate.

Protocol for binding dissociation:

1. Add 5 µl GST-cGIRK1.
2. Add 5 µl biotin-Gβ1γ2.
3. Add 5 µl anti-GST-acceptor beads.
4. Incubate 30 minutes at RT.
5. Add 5 µl Gβ1γ2.
6. Incubate 10 minutes at RT.
7. Add 5 µl streptavidin-donor beads.
8. Incubate 10 minutes at RT.
9. Read plate.

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