

Beads*

- Streptavidin-coated Donor beads
- Unconjugated Donor beads
- Unconjugated Acceptor beads
- Conjugation kit**

Fusion Tag Detection Kits***

- GST (Glutathione-S-Transferase) detection kit
- DIG (Digoxin/Digoxigenin) detection kit
- FITC (Fluorescein) detection kit
- HIS₆ (6-Histidine) detection kit
- Histidine (Nickel chelate) detection kit
- c-myc detection kit
- HA (Hemagglutinin) detection kit
- FLAG™ (M2) detection kit

IgG Detection Kits***

- Goat IgG detection kit
- Human IgG detection kit
- Mouse IgG detection kit
- Rabbit IgG detection kit
- IgG (Protein A) detection kit

GPCR Functional Assay Kits

- cAMP assay kit****
- IP₃ assay supplement***** (Requires GST detection kit)

Phosphotyrosine Assay Kits***

- Phosphotyrosine (PY20) assay kit
- Phosphotyrosine (PT66) assay kit
- Phosphotyrosine (P-Tyr-100) assay kit

* Available in 1, 5 and 50 mg quantities. 1 mg translates into 2,000 assay points based on a 25 µL reaction volume and final bead concentration of 20 µg/mL.

** 2 mg ea. streptavidin-coated Donor beads / unconjugated Acceptor beads.

*** Available in 500, 10,000 and 50,000 assay point quantities based on a 25 µL reaction volume and final bead concentration of 20 µg/mL.

**** Available in 500, 10,000 and 50,000 assay point quantities based on a 25 µL reaction volume and final donor and acceptor bead concentration of 20 µg/mL and 15 µg/mL, respectively.

***** Available in 500 and 10,000 assay point quantities based on a 50 µL reaction volume and final bead concentration of 10 µg/mL.

Microplates and Microplate Analyzers

Fusion™-α and **AlphaQuest®-HTS** microplate analyzers and **Optiplate™ microplates** are optimized to measure AlphaScreen signal and are available from Packard BioScience Company.

For more information please contact your local Packard BioScience office or visit our website at

www.packardbioscience.com

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AlphaScreen™ Phosphotyrosine (PT66) Assay Kit

Product Information & Titration Curve Protocol

Product Information

AlphaScreen™ Phosphotyrosine (PT66) Assay Kit

Catalog No.: **6760602C**
Kit size: **500 assay points***

* The number of assay points is based on a 25 µL reaction volume and a final bead concentration of 20 µg/mL.

Kit Components

Anti-phosphotyrosine (PT66) Acceptor Beads

1 tube containing 50 µL PT66 Acceptor beads at 5 mg/mL in 25 mM HEPES 100 mM NaCl, pH 7.4.

Streptavidin Donor Beads

1 tube containing 50 µL streptavidin Donor beads at 5 mg/mL in 25 mM HEPES 100 mM NaCl, pH 7.4.

Biotinylated-LCK-P

1 tube containing 50 µL biotinylated-LCK-P at 500 nM in 25 mM HEPES pH 7.4.

10x Buffer

1 tube containing 1.5 mL 250 mM HEPES, 1 M NaCl, pH 7.4.

Note: 0.05% Proclin-300 is added as a preservative to all components.

Product QC/QA

Donor and Acceptor beads are controlled to meet chemical composition, dextran loading and conjugation requirements.

AlphaScreen lot-to-lot consistency is confirmed by a competition and titration assay to meet defined specifications under standard conditions.

Titration Curve Protocol

Note: This protocol provides a means to verify kit performance and is not representative of an assay. Sufficient biotinylated-LCK-P and 10x buffer is provided to perform 3 titration curves in triplicate as described. To perform an actual assay with the AlphaScreen Phosphotyrosine (PT66) Assay Kit, please refer to the appropriate application notes and protocol books.

Materials & preparation:

1x Buffer

Add 500 µL 10x buffer to 4.5 mL Milli-Q® H₂O (or equivalent). Add 5 mg BSA (0.1% final concentration) and adjust pH to 7.4.

Anti-Phosphotyrosine (PT66) Acceptor beads (Provided)

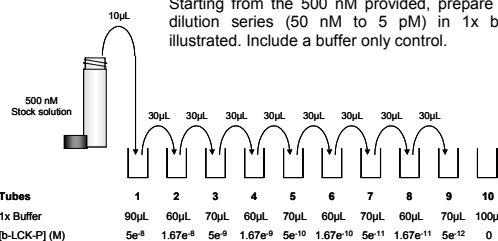
Add 4 µL Anti-Phosphotyrosine (PT66) Acceptor beads to 196 µL 1x buffer.

Streptavidin Donor beads (Provided)

Add 4 µL streptavidin Donor beads to 196 µL 1x buffer.

Biotinylated-LCK-P (Provided)

Starting from the 500 nM provided, prepare a ½ log dilution series (50 nM to 5 pM) in 1x buffer as illustrated. Include a buffer only control.



Method:

To a 384-well white opaque Optiplate™-384 NEW* (Packard BioScience part number 6007290):

1. Add 15 µL biotinylated-LCK-P dilutions (tubes 1 through 10).
2. Add 5 µL PT66 Acceptor bead.
3. Add 5 µL streptavidin Donor beads.

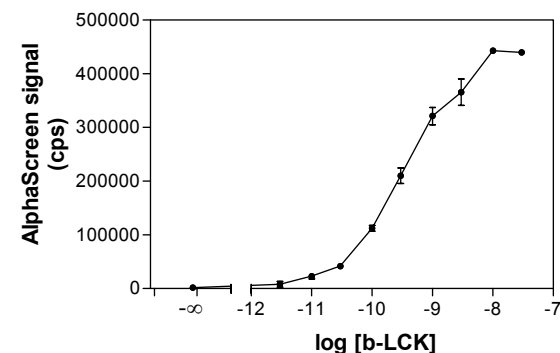
Incubate** in the dark*** at room temperature for at least 60 minutes and analyze on either the AlphaQuest™-HTS or Fusion™-α microplate analyzer.

* It is recommended that the following steps be performed in triplicate.

** Plates can be covered with TopSeal™ (Packard BioScience part number 6005185) adhesive sealing film to reduce evaporation during incubation.

*** Plates can be covered by another microplate.

Result:



The above graph illustrates a typical biotinylated-LCK-P titration curve performed as described (25 µL final volume in a 384-well microplate) at room temperature (22-23°C) and 1 hour incubation. Total signal will vary with ambient temperature and incubation time. Signal is stable with an overnight incubation. For consistent results, the same incubation times and temperature should be used for each plate.

Final concentrations of reagents in the assay are 20 µg/mL Donor beads, 20 µg/mL Acceptor beads, and biotinylated-LCK-P at 30 nM, 10 nM, 3 nM, 1 nM, 0.3 nM, 0.1 nM, 0.03 nM, 0.01 nM, 3 pM, and 0 M.

Precautions

1. AlphaScreen beads are light sensitive.

- Take care to not expose beads to bright light. Beads are best handled under subdued or green filtered light conditions.
- Incubation steps involving the beads should be performed in the dark. Plates can be covered by another microplate to minimize the effect of light.

2. Storage.

- Beads should be stored in the dark at 4°C.
- To ensure product longevity, unused AlphaScreen reagents should be stored in the original packaging, at the original concentration and at the recommended temperature.
- Do not store reagents containing BSA for more than 1 day at 4°C.

3. Interfering compounds.

- For information on potential interfering compounds please refer to application note ASC-012.