

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
- HIS₆ (6-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

For technical assistance please contact:

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AlphaScreen™ Conjugation Kit

Product Information & Conjugation Protocol

Product Information

AlphaScreen™ Conjugation Kit

Catalog No.: **6760000K**
Quantity: **2 x 2 mg***

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

Components

Streptavidin Donor beads

2 tubes each containing 200 µL of beads at 5 mg/mL in 25mM HEPES 100mM NaCl pH 7.4.

Unconjugated Acceptor beads

2 tubes each containing 50 µL of beads at 20 mg/mL in 0.1 M MES buffer pH 6.0.

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

Product QC/QA

Unconjugated Donor and Acceptor beads are controlled to meet chemical and dextran loading requirements.

Lot to lot consistency is confirmed by producing and mixing Streptavidin-coated Donor beads with Biotin-coated Acceptor beads to yield a defined signal under standard conditions.

Recommendation

AlphaScreen streptavidin Donor and conjugated Acceptor beads should be used at a concentration of 20 µg/mL for best results.

Conjugation Protocol

Note: The following procedure is recommended only as a general guideline for bead conjugation to IgG molecules. It is highly recommended that conditions are further optimized as required for the application for which the beads are intended.

Materials:

Aldehyde beads (Provided)

Sodium cyanoborohydride solution

Dissolve 25 mg of sodium cyanoborohydride (Aldrich part number 15,615-9 or equivalent) in 1 mL Milli-Q® H₂O or equivalent.

Note: Prepare fresh.

MES buffer (0.1 M pH 6.0)

Dissolve 2.13g MES (2-[N-Morpholino]ethanesulfonic Acid) (Sigma part number M-5287 or equivalent) in 80 mL Milli-Q® H₂O or equivalent. Adjust the pH to 6.0 with 10 N NaOH and make up to 100 mL total volume. Filter through a 0.2 µm filter.

1% Tween-20

Dilute 10% Tween-20 (Pierce part number 28320 or equivalent) 1/10 in MES buffer.

CMO solution (0.3 M pH 5.0)

Dissolve 65 mg CMO (carboxymethoxylamine hemihydrochloride) (Aldrich part number C1,340-8 or equivalent) in 1 mL Milli-Q® H₂O or equivalent. Adjust the pH to 5.0 with 10 N NaOH.

Note: Prepare fresh.

TRIS buffer (0.1 M pH 8.0)

Dissolve 1.21g Tris (ICN part number 819638 or equivalent) in 80mL deionised H₂O. Adjust the pH to 8.0 with HCl and make up to 100mL total volume. Filter through a 0.2µm filter.

Method:

Conjugation

- To each tube (1 mg beads) provided add:
 - 12.5 µL 1% Tween-20
 - 0.05 mg antibody
(This will provide a 20:1 bead:antibody wt:wt ratio. As antibodies are available in different concentrations depending on the supplier, 50 µL of a 1 mg/mL solution is recommended)
 - 10µL Sodium cyanoborohydride solution
- Make up to a total volume of 200 µL with 0.1 M MES pH 6.0.
Incubate in the dark for 48 hours at 37°C.

Blocking

- Add 10 µL CMO solution.
Incubate in the dark at 37°C for 1 hour.

Method (continued):

Purification

- Add 190 µL 0.1 M Tris buffer pH 8.0.
- Centrifuge at 13,000 g for 30 min at 4°C.
- Remove the supernatant with a micropipette.
- Add 1 mL 0.1 M Tris buffer pH 8.0 to pellet.
- Vortex and sonicate*.
- Centrifuge at 13,000 g for 30 min at 4°C.
- Repeat steps 3 through 6.
- Add 200 µL of an appropriate buffer** (5 mg/mL final concentration).
- Vortex and sonicate*.
- Store in the dark at 4°C

* Sonicate on ice at 10 pulses of 1 second (0.5 second ON/0.5 second OFF) using a Misonix XL sonicator (Model CL4) and HS419 microtip probe.

** Either PBS or 25 mM Hepes, 100 mM NaCl, pH 7.4 is recommended.

Precautions

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