

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™ Unconjugated Acceptor Beads

Product Information & Conjugation Protocol

Product Information

Conjugation Protocol

AlphaScreen™

Unconjugated Acceptor Beads

Catalog No.: **6762003**

Quantity: **1 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

Unconjugated Acceptor beads

1 tube 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 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 Acceptor beads should be used at a concentration of 20 µg/mL for best results.

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 the 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.