

Caution: For Laboratory Use. A research reagent for research purposes only

AlphaScreen® SureFire HV™ IgG (Protein A) Detection Kit, 96-well format

Product No.: 6760617HV

Lot No.: 678-333-A

Material Provided

Format: 100 Assay Points

Note: The reagents provided in this kit are sufficient for 100 assay points when following the AlphaScreen® SureFire HV™ protocol for 96-well format assays.

Manufacturing Date: August 16, 2011

Kit Components:

Component	6760617HV
Protein A Acceptor beads at 5 mg/mL in 0.1M Tris, 0.05% Proclin-300, pH 8.0	1 × 20 µL (6760136HV)
Streptavidin Donor beads at 5 mg/mL in 0.1M Tris, 0.05% Proclin-300, pH 8.0	1 × 35 µL (6760007HV)
Biotinylated rabbit IgG at 0.05 µM in 1X PBS, 0.1% BSA, 0.05% Proclin-300, pH 7.2	1 × 50 µL (6760264)
10X Buffer : 1M Tris, 0.1% Tween-20, 0.05% Proclin-300, pH 8.0	1 × 1.5 mL (6760028G)

Product Information

Antibody/Protein: Protein A Acceptor beads are coated with Protein A. Protein A binds the Fc region of many IgG.

Stability: This product is stable for at least **9 months** from the manufacturing date if used and stored under recommended conditions.

Storage Conditions: Store undiluted at 4°C protected from light. Freeze-thaw is not recommended and can cause the beads to form aggregates.

Recommended use: AlphaScreen® Donor beads are **light sensitive** and should be handled under subdued or green filtered light conditions (< 100 Lux). Vortex beads prior to use.

For additional information on running AlphaScreen® assays or on potential interfering compounds, please visit our website: www.perkinelmer.com/AlphaTech

Quality Control

AlphaScreen® maximum signal, minimum signal and EC₅₀ are determined using a biotinylated rabbit IgG (rlgG) titration assay performed on an EnVision® HTS Alpha detection instrument. We certify that these results meet our requirements.

<u>TEST</u>	<u>RESULTS</u>
Maximum signal	804 996 cps
Minimum signal	412 cps
EC₅₀	0.036 nM

The Quality Control protocol provides a method to verify kit performance. It is not representative of an AlphaScreen® SureFire HV™ assay in 96-well format. Sufficient biotinylated probe and 10X buffer is provided in this kit to perform three titration curves in triplicate in 384-well format as described:

- 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 8.0.
- Acceptor Beads:** Add 5 µL Protein A Acceptor beads to 245 µL 1X buffer.
- Donor beads:** Add 5 µL Streptavidin Donor beads to 245 µL 1X buffer.
- Biotinylated-probe:** From the 0.05 µM biotinylated rlgG stock, prepare a half-log dilution series (15 nM to 1.5 pM) in 1X buffer. Include a buffer only control.

Titration Protocol:

To a white opaque 384-well OptiPlate™ microplate:

1. Add 5 µL biotinylated rlgG dilutions (from lowest to highest concentrations).
2. Add 10 µL 1X buffer.
3. Add 5 µL Protein A Acceptor beads.

Incubate in the dark at room temperature for 30 minutes.

4. Add 5 µL Streptavidin Donor beads.

Incubate in the dark at room temperature for 60 minutes and analyze on a reader equipped with an Alpha detection module such as the EnVision® or EnSpire® multilabel plate readers.

Note: Alternatively, a single titration curve in 96-well format could be performed by doubling volumes of addition (final volume of 50 µL) in a white opaque 96-well OptiPlate™ microplate.

Product Typical Data

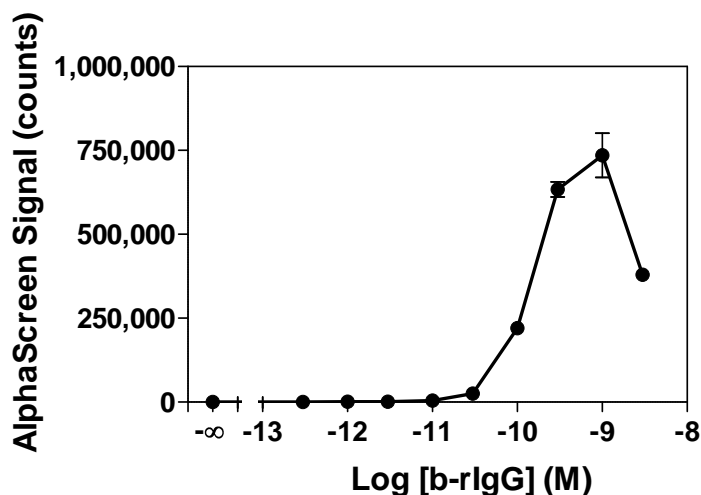


Figure 1: Biotinylated-probe titration assay. Biotinylated rIgG (b-rlgG) was titrated in a final volume of 25 μ L in 384-well format. Signal was detected on an EnVision[®] HTS Alpha.

Note: AlphaScreen[®] signal will vary depending on instrument detection protocol, incubation temperature and incubation time.

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/AlphaTech

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