

AlphaScreen®

Histidine (Nickel Chelate) Detection Kit

Product number: 6760619C

Lot Number: 3292774

Product Format: 6760619C: 500 assay points

6760619M: 10 000 assay points

6760619R: 50 000 assay points

The number of assay points is based on an assay volume of 25 µL in 384-well assay plates using a final bead concentration of 20 µg/mL.

Manufacturing date: 06/21/2024

Document version: 1

Kit Components

| Component | 6760619C | 6760619M | 6760619R |
|--|--------------------------|--------------------------|--------------------------|
| Nickel Chelate Acceptor Beads at 5 mg/mL in 25 mM Hepes, 100 mM NaCl, 0.05% Kathon, pH 7.4 | 1 x 50 µL (6760140) | 1 x 1 mL (6760141) | 1 x 5 mL (6760141B) |
| Streptavidin Donor Beads at 5 mg/mL in 25 mM Hepes, 100 mM NaCl, 0.05% Kathon, pH 7.4 | 1 x 50 µL (6760001) | 1 x 1 mL (6760002) | 1 x 5 mL (6760002B) |
| Biotinylated-(His) ₆ at 0.5 µM in 25mM Hepes, 0.05% Kathon, 0.1% BSA, pH 7.4 | 1 x 50 µL (6760302) | 1 x 50 µL (6760302) | 1 x 50 µL (6760302) |
| 10x Buffer : 250 mM Hepes, 1 M NaCl, 0.05% Kathon, pH 7.4 | 1 x 1.5 mL (6760020G) | 1 x 1.5 mL (6760020G) | 1 x 1.5 mL (6760020G) |

Product Information

Antibody/Protein: The Nickel Chelate Acceptor beads are coated with chelated nickel which binds (HIS)₆ tagged proteins.

Stability: This kit is stable for at least 12 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

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

Recommended Use: AlphaScreen® Donor beads are light-sensitive. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters) can be applied to light fixtures.

Quality Control

Alpha maximum signal, minimum signal and EC50 are determined using a biotinylated-(His)₆ titration assay performed on an EnVision® instrument. We certify that these results meet our requirements.

| | |
|--------------------|---------------|
| Maximum Signal:: | 733383 counts |
| Minimum Signal: | 439 counts |
| EC ₅₀ : | 0.86 nM |

Recommended Assay Conditions

Note: This protocol provides a method to verify kit performance and is not representative of an assay. Sufficient biotinylated-probe and 10x buffer is provided to perform 3 titration curves in triplicate as described.

| | |
|---------------------|--|
| 1x Buffer: | Add 500 µL 10x buffer to 4.5 mL Milli-Q® H ₂ O (or equivalent). Add 5 mg BSA (0.1% final concentration) and adjust pH to 7.4. |
| Acceptor Beads: | Add 5 µL Nickel chelate Acceptor beads to 495 µL 1x buffer. |
| Donor beads: | Add 5 µL Streptavidin Donor beads to 495 µL 1x buffer. |
| Biotinylated-probe: | From the 0.5 µM biotinylated-(His) ₆ , prepare a ½ log dilution series (0.5 µM to 50 pM) in 1x buffer. Include a buffer only control. |

Titration Protocol

To a white opaque OptiPlate-384:

- 1) Add 5 µL biotinylated-(His)₆ dilutions (from lowest to highest concentration).
- 2) Add 10 µL of Nickel chelate Acceptor beads.

Incubate in the dark at room temperature for 30 minutes

- 3) Add 10 µL of Streptavidin Donor beads.

Incubate in the dark at room temperature for 60 minutes and analyze on your Alpha capable detection reader.

Typical Product Data

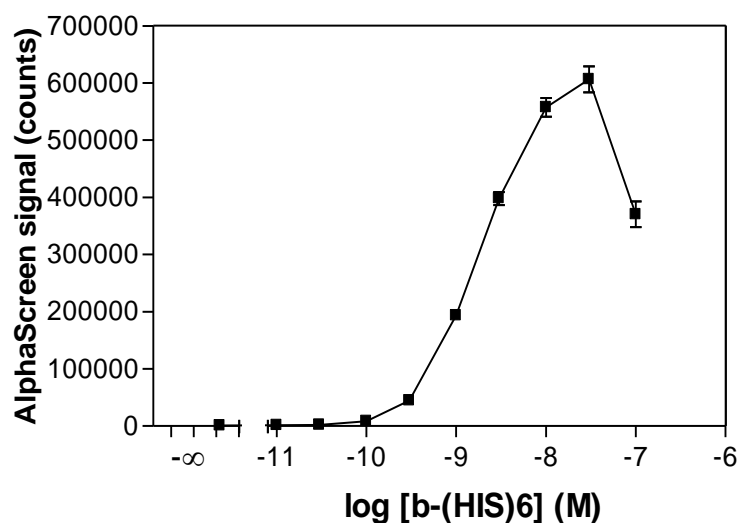


Figure 1: Biotinylated-probe titration assay 384-well biotinylated-(His)6 titration curve (25 μ L final volume; Reader: Envision). Note: Alpha signal will vary depending on instrument detection protocol, incubation temperature and incubation time.

Please visit our website for additional information on AlphaLISA technology at www.revivity.com

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