revvity

CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

Conjugation Kit

Product number: 6760000K **Lot Number:** 3225063

Product Format: 6760000K 2 x2mg

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

Manufacturing date: 11/6/2023 **Document version:** 1

Kit Components

Component	6760000K
Unconjugated Acceptor Beads at 20 mg/mL in 0.1M MES, pH 6.0	2 x 50 µL (6762001)
Streptavidin Donor Beads at 5 mg/mL in 25mM Hepes, 0.1M NaCl, 0.05% proclin, pH 7.4	2 x 200 µL (6760002S)

Product Information

Stability: This product is stable for at least 12 months from the manufacturing date if used and stored under recommended conditions.

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

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 (Sigma #15,615-9 or equivalent) in 1 mL Milli-Q® H_2O or equivalent. **Note**: Prepare fresh.

MES buffer (0.1 M pH 6.0): Dissolve 2.13g MES (2-[N-Morpholino]ethanesulfonic Acid) (Sigma # M-5287 or equivalent) in 80 mL Milli-Q® H_2O 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 #28320 or equivalent) 1/10 in MES buffer.
CMO solution (0.3 M pH 5.0): Dissolve 65 mg CMO (carboxymethoxylamine hemihydrochloride) (Sigma #C1,340-8 or equivalent) in 1 mL Milli-Q® H_2O 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 #819638 or equivalent) in 80mL deionised H_2O. Adjust the pH to 8.0 with HCl and make up to 100mL total volume. Filter through a 0.2µm filter.

Conjugation:

1. 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
2. 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.

Purification:

1. Add 190 µL 0.1 M Tris buffer pH 8.0.
2. Centrifuge at 13,000 g for 30 min at 4°C.
3. Remove the supernatant with a micropipette.
4. Add 1 mL 0.1 M Tris buffer pH 8.0 to pellet.
5. Vortex and sonicate*.
6. Centrifuge at 13,000 g for 30 min at 4°C.
7. Repeat steps 3 through 6.
8. Add 200 µL of an appropriate buffer** (5 mg/mL final concentration).
9. Vortex and sonicate*.
10. 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.*

Please visit our website for additional information on AlphaScreen technology at www.revvity.com

6760000K-R Rev 01

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revvity.com