General protocol for the preparation of antibody-conjugated EYRAbeads

This protocol provides general guidelines for covalently coupling antibodies to Mabtech EYRAbeads for use in multiplex bead-based immunoassays. To covalently couple antibodies to carboxylated magnetic beads for use in multiplex bead-based immunoassays.

Materials needed:

- Mabtech EYRAbeads
- Antibody to be conjugated
- Sulfo-NHS: e.g., Thermo Scientific™ Sulfo-NHS (N-hydroxysulfosuccinimide), No-Weigh™ Format, 10 x 2 mg (A39269)
- EDC: e.g., Thermo Scientific™ Pierce™ EDC, No-Weigh™ Format, 10 x 1 mg (A35391)
- Activation buffer: e.g., MES buffer, pH 6.0–6.2
- Conjugation buffer: e.g., acetate or MES buffer, pH 5.0-6.0
- Quenching buffer: e.g., PBS with 100 mM ethanolamine-HCl, pH 8
- Storage buffer: e.g., PBS, 1% BSA, kathon or azide, pH 7.4, with or without 0.1% Tween
- 1.5, 2, or 5 ml microcentrifuge tubes
- Desalting columns: e.g., PD-10, PD SpinTrap G-25, or Zeba Spin Desalting Columns 7K
- Spectrophotometer or similar for concentration determination
- Magnetic rack capable of holding appropriate tubes: e.g., Millipore 8 tube magnet or BioRad 16 tube magnet
- Tube rotator, preferably end-over-end mixer
- Cell/bead counter or flow cytometer suitable for bead counting
- Vortex and optional water bath sonicator for better dispersion in initial steps

Important notes:

- Always **protect beads from bright light**; keep tubes covered with aluminum foil when not in use.
- Perform all procedures in a clean lab environment with lights off or dimmed.
- Perform all bead washing steps using a **magnetic rack**.
- **Do not keep beads on the magnet for longer than necessary** - remove the tubes as soon as the supernatant is cleared to avoid bead aggregation. 2 minutes is often enough for smaller scale couplings
- **Mix beads thoroughly** by vortexing for 30 seconds, followed by sonication (if available) for 5-10 seconds before pipetting (within 30 seconds of vortexing).

Table 1. Recommended volumes for washes, reagents and buffers.

Number of EYRA-beads (millions)	Buffer-exchanged antibody volume (ml)	Tube size	Wash volume (ml)	Volume for activation (ml)	S-NHS & EDC 50 mg/ml (µl of each)	Conjugation buffer volume (ml)	Total conjugation volume (ml)
1-4	0.3	1.5 ml	0.8	0.5	50	0.3	0.6
5-8	0.6	5 ml	1.6		50	0.6	1.2
9-12	0.9	5 ml	2.4		50	0.9	1.8
13-16	1.2	5 ml	2.4		50	1.2	2.4

I. Preparation of capture antibody

1. **Buffer exchange** the capture antibody into conjugation buffer using a desalting column.
   - Note: Do not keep antibodies in conjugation buffer for longer than 2 hours before conjugation.
2. If needed, **concentrate** the mAb to achieve a concentration of 0.05 mg/ml (preferably use a high-concentration stock for buffer exchange).
3. After buffer exchange:
   - Filter the mAb through a **0.22 µm** sterile filter.
   - Measure the antibody concentration (e.g., by absorbance at 280 nm).
4. **Dilute** the mAb in conjugation buffer to a concentration that results in 4 µg antibody/million beads in the specified volume of buffer-exchanged antibody in Table 1. For example, if conjugating 1 million beads, the buffer-exchanged antibody should be diluted to 13.3 µg/ml in 0.3 ml with a total conjugation volume of 0.6 ml.

II. Preparation of EYRAbeads

5. **Mix beads thoroughly** by vortexing for 30 seconds, followed by sonication (if available) for 5-10 seconds before pipetting (within 30 seconds of vortexing).
6. **Transfer** the desired number of EYRAbeads to a suitable tube (see Table 1).
7. **Add activation buffer** to reach the recommended wash volume, according to Table 1.
8. **Wash** the EYRAbeads four times with the recommended wash volume of **activation buffer**, vortexing thoroughly after each wash.
9. **Resuspend** the EYRAbeads in the recommended resuspension volume of activation buffer, see Table 1.

III. Activation of EYRAbeads

Note: All steps should be performed in a chemical hood.

10. **Dissolve** sulfo-NHS and EDC freshly in activation buffer:
    - Sulfo-NHS: dissolve to 50 mg/ml in activation buffer.
    - EDC: dissolve to 50 mg/ml in activation buffer.
11. **Add** the recommended volume of sulfo-NHS and EDC (see Table 1) to the bead suspension prepared in Step II.
12. **Incubate** on a tube rotator, preferably in oscillating mode, at **RT for 15 min**.

IV. Conjugation of capture antibody to activated EYRAbeads

13. **Remove the activation buffer** using the magnetic rack.
14. **Wash** the EYRAbeads four times with conjugation buffer, vortexing thoroughly after each wash.
15. **Resuspend** the EYRAbeads in the recommended volume of conjugation buffer, according to Table 1.
16. **Add the buffer-exchanged capture antibody** in the same volume as the conjugation buffer to the bead suspension (1:1) and mix immediately by pipetting.
17. **Incubate** on a tube rotator, preferably in oscillating mode, at **room temperature overnight.**

V. Quenching of conjugated EYRAbeads

18. **Wash the conjugated EYRAbeads with the magnet as described previously:**
    - Once with conjugation buffer
    - Three times with quenching buffer (vortex thoroughly after each wash).
19. **Resuspend** the EYRAbeads in the recommended wash volume of quenching buffer.
20. **Incubate** on a tube rotator, preferably in oscillating mode, at **RT for 60 min.**

VI. Preparation for storage

21. **Wash the quenched** EYRAbeads three times with freshly filtered storage buffer, vortexing thoroughly after each wash.
22. **Resuspend** the EYRAbeads in a suitable final volume of storage buffer, e.g., 2×10^6 beads/ml.
23. **Count the EYRAbeads** using a cell/bead counter or suitable flow cytometry instrument.
24. **Aliquot** into dark vials and store at 4-8°C.

26/01/2026