VERSION 1

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 We use this protocol and it's working

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Luminex Bead Conjugation V.1

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ABSTRACT

Protocol for amine-coupling of proteins to magnetic Luminex beads

Quantity as written: 1×10^6 beads under typical yields (sufficient for 2000 wells assuming 500 beads/well)

Needs a good qualification section!

MATERIALS

STEP MATERIALS

⊗ EDC (no-weigh formulation) **Thermo Scientific Catalog #A35391**

⊗ sulfo-NHS (no-weigh format) **Thermo Scientific Catalog #A39269**

⊗ PBS-TBN **Teknova Catalog #P0210**

⊗ PBS-TBN **Teknova Catalog #P0210**

Activation

1 Resuspend beads by briefly vortexing; dispense 100 µL (contains approx. 1.25 million beads)

2 BEAD SEPARATION BLOCK

2.1 Collect volume by pulse centrifugation 100 x g 00:00:03

2.2 Place on magnetic tube rack, shielded from light 00:01:00

Note

Protocol tip

Partially open the microcentrifuge tube before placing in the rack. This allows them to be opened on-rack with minimal disruption of the bead pellet that's held in place only by the magnet.

2.3 Remove 90 µL of supernatant (while still on-rack) using a pipette

Note

n.b.

This leaves a 10 µL excess that allows the removal of supernatant at all subsequent steps to use the same volume that was added immediately prior.

3 WASH BLOCK

3.1 Resuspend in 100 µL 1x PBS; vortex briefly to mix

3.2 Collect volume by pulse centrifugation 100 x g 00:00:03

3.3 Place on magnetic tube rack, shielded from light 00:01:00

3.4 Remove supernatant using a pipette

4 Resuspend beads in a solution containing:

- 100 millimolar (mM) sodium phosphate (pH 6.2) 80 µL
- 12.5 mg/mL (approx. 65 millimolar (mM)) in sodium phosphate (pH 6.2)
5 µL
- 12.5 mg/mL (approx. 58 millimolar (mM)) sulfo-NHS in phosphate (pH 6.2)
5 µL

EDC (no-weigh formulation) **Thermo Scientific Catalog #A35391**

sulfo-NHS (no-weigh format) **Thermo Scientific Catalog #A39269**

5 Mix tubes end-over-end, shielded from light Room temperature 00:20:00

Coupling

- 6 go to step #2 (separation block) for removal of supernatant
- 7 Resuspend beads in 25 µg/mL protein of interest 250 µL, made in 1x PBS
- 8 Mix tubes end-over-end, shielded from light Room temperature 02:00:00

Blocking + Washes

- 9 go to step #3 (wash block) for a **total of 3** washes using 400 µL 1x PBS-TBN

Note

n.b.

DO NOT use P1000 for removing supernatant as the wider tip and faster flow tends to lead to greater bead loss. Instead, use a P200 twice.

PBS-TBN Teknova Catalog #P0210

- 10 Perform final resuspension in 250 µL 1x PBS-TBN

PBS-TBN Teknova Catalog #P0210

- 11 Will the beads be used immediately?

Counting

- 12 Dilute beads by 1:10 and 1:100 in 1x PBS
- 13 Measure concentration of each dilution by cell counter

Expected result

Typical yields are approx. 1×10^6 beads, or 4×10^6 beads/mL