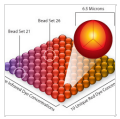

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Luminex Bead Conjugation

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ABSTRACT

Protocol for amine-coupling of proteins to magnetic Luminex beads

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

Needs a good qualification section!

STEPS MATERIALS

NAME	CATALOG #	VENDOR
EDC (no-weigh formulation)	A35391	Thermo Scientific
sulfo-NHS (no-weigh format)	A39269	Thermo Scientific
PBS-TBN	P0210	Teknova
PBS-TBN	P0210	Teknova

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**

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

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 Milimolar (mM)** sodium phosphate (pH 6.2) **80 µl**
- **12.5 mg/mL** (approx. **65 Milimolar (mM)**) in sodium phosphate (pH 6.2) **5 µl**
- **12.5 mg/mL** (approx. **58 Milimolar (mM)**) sulfo-NHS in phosphate (pH 6.2) **5 µl**

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

sulfo-NHS (no-weigh format)
by 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

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
by Teknova
Catalog #: P0210

10 Perform final resuspension in **250 µl** 1x PBS-TBN

PBS-TBN
by Teknova
Catalog #: P0210

11 Will the beads be used immediately?

Counting

Dilute beads by 1:10 and 1:100 in 1x PBS

12

13 Measure concentration of each dilution by cell counter

Typical yields are appox. 1 x 10^6 beads, or 4 x 10^6 beads/mL