



LEGENDplex™ Carboxyl Bead Conjugation Protocol

This protocol describes the conjugation of activated beads to targets that contain amines, usually proteins such as antibodies, streptavidin, and similar molecules.

This is not a universal protocol, and it may not work with some molecules. Each biological molecule is unique and depending on its biochemical characteristics (for example isoelectric point) the protocol may need to be optimized. Please verify the efficiency of conjugations with an appropriate method if necessary.

Materials required:

1. LEGENDplex™ Carboxyl Beads
2. 1.5 mL microcentrifuge tubes
3. Table top microcentrifuge capable of spinning at 12,000 rpm
4. Vortex
5. Rotary shaker with microcentrifuge tube holders, capable of shaking at 1000 rpm
6. Adjustable volume pipettes (20 to 1000 µL)
7. Coupling Buffer (See notes regarding solutions and buffers used in the protocol)
8. EDC solution (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride)
9. Sulfo-NHS (N-hydroxysulfosuccinimide)
10. Blocking Buffer
11. Storage Buffer

Notes:

1. **The beads are light sensitive and should be protected from light as much as possible throughout the coupling procedure.** All reagents should be warmed up to room temperature before use.
2. Coupling buffer: As no single buffer is ideal for every ligand, it may vary depending on the protein to be conjugated. Acidic buffers such as 0.1 M phosphate pH 6.0 or 0.05 M MES buffer pH 5.0 are the most commonly used.
3. Several providers of chemical reagents such as Sigma Aldrich and Thermo Scientific offer EDC and sulfo-NHS ready to use. **These solutions should be prepared immediately before use, and kept protected from light.**
4. Blocking buffer: PBS containing up to 1% BSA, pH 7.4.
5. Storage buffer: PBS containing 0.01% Tween® 20, 0.05% NaN₃, and 0.1% BSA, pH 7.4.

Preparation of Carboxyl Beads

1. Transfer 10^7 LEGENDplex™ Carboxyl Beads to a 1.5 mL microcentrifuge tube.
2. Centrifuge the tube at 12,000 rpm in a table top microcentrifuge for 3 minutes to pellet the beads.
3. Discard the supernatant by aspiration with a pipette.
4. Add 400 μ L Coupling Buffer and resuspend the beads completely by vortexing.
5. Repeat step 2, 3 and 4.
6. Repeat step 2 and 3.
7. Resuspend beads completely in 160 μ L of Coupling Buffer.

Bead Activation with EDC and Sulfo-NHS

1. Prepare 50 mg/mL EDC solution and 50 mg/mL Sulfo-NHS solution in coupling Buffer.
2. Add 20 μ L Sulfo-NHS solution and 20 μ L EDC solution to the beads prepared above, and mix completely by vortexing for 5 seconds.
3. Incubate for 20 minutes at room temperature with agitation, rotary shaker at 1000 rpm.
4. Centrifuge the tube at 12,000 rpm in a table top microcentrifuge for 3 minutes to pellet the beads.
5. Discard the supernatant by aspiration with a pipette.
6. Add 400 μ L Coupling Buffer and resuspend the beads by vortexing.
7. Repeat step 4, 5 and 6.
8. Repeat step 4 and 5.
9. Resuspend beads completely in 180 μ L of Coupling Buffer.

Protein Coupling to Beads

1. Determine the amount of protein to be conjugated. Use between 1 – 10 μ g/ 10^6 beads, depending on the molecular weight of the protein.

Note: The protein sample must be free of carboxyl or amine bearing-compounds such as BSA, Tris, Glycine, and similar products. The optimal amount of protein to be used may need to be titrated.

2. Add the desired amount of protein in a volume of circa 20 μ l to the tube containing the activated beads. Mix well by vortexing for 5 seconds.
3. Incubate for 2 hours at room temperature with agitation, rotary shaker at 1000 rpm.
4. Centrifuge the tube at 12,000 rpm in a table top microcentrifuge for 3 minutes to pellet the beads.
5. Discard the supernatant by aspiration with a pipette.

Blocking and Storage

1. Resuspend the beads in Blocking Buffer and incubate for 1hour at room temperature with agitation, rotary shaker at 1000 rpm, to block any remaining active site.
2. Centrifuge the tube at 12,000 rpm in a table top microcentrifuge for 2 minutes to pellet the beads.
3. Resuspend in Storage Buffer.

BioLegend Inc.

8999 BioLegend Way

San Diego, CA 92121

Tel: 1.858.768.5800

Tel: US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)

Technical Service Email: techserv@biolegend.com

biolegend.com