Protocol

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Coupling Protocol for LodeStars Carboxyl Magnetic Beads, EDC Mediated (One Step)

Introduction

The following protocol provides users with guidance for the successful coupling of biomolecules to Agilent LodeStars magnetic beads with carboxyl surfaces. This general protocol describes steps for coupling of biomolecules, but Agilent recommends further optimization to achieve maximum performance in your application.

The typical strategy for ligand coupling is the formation of an amide bond between a primary amino group of the ligand and the carboxylic acid groups on the surface of the magnetic particles, mediated by carbodiimide activation.

EDC protein-binding protocol

Material required to perform the coupling protocol

- 0.1 M MES (2-[morpholino]ethanesulfonic acid), pH 5
  - Alternate pH conditions can be explored as part of optimization
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide)
- Reverse osmosis or ultrapure water
- Phosphate-buffered saline (PBS): 0.1 M sodium phosphate, pH 7.4
  - **Tip:** For long storage periods, it is recommended to add 0.1 %w/v sodium azide to avoid microbial growth
- PBS-Tween buffer (PBST): 0.1 M sodium phosphate, pH 7.4, 0.1 %w/v Tween-20.
- 2 mL microcentrifuge tubes
- Magnetic separator
- Microcentrifuge
- Vortex mixer
- Bottle roller

Before starting with the binding protocol

- Check the expiration date of the buffers (6 months since preparation).
- Allow the buffers and reagents to reach room temperature.
- Disperse the beads using a bottle roller for a minimum of 2 hours for volumes above 250 mL or 1 hour for smaller volumes. Non-homogeneous dispersion could result in an incorrect quantity of beads being used in conjugation or aggregations, causing incomplete coupling. For an optimal bead dispersion, Agilent recommends rolling beads at room temperature overnight (~16 hours).
- Prepare the protein solution: 0.1 M MES at pH 5.0 with appropriate amounts of proteins of interest. For LodeStars carboxyl beads, saturation concentrations for streptavidin are 30 to 35 µg of protein/mg of LodeStars beads, whereas IgG is approximately 20 to 30 µg/mg. For LodeStars High Bind carboxyl beads, saturation concentrations for streptavidin are 50 to 60 µg of protein/mg of LodeStars High Bind beads, whereas concentration for IgG is approximately 45 to 55 µg/mg.

Procedure

1. Aliquot 10 mg of LodeStars beads (approximately 333 µL) or LodeStars High Bind beads (approximately 200 µL) into a 2 mL microcentrifuge tube. **Note:** The exact volume needed for 10 mg must be calculated from the certificate of analysis.
2. Place the tube in a magnet to separate the beads from the solution. Once the separation has been completed, aspirate the supernatant, then remove the tube from the magnet.
3. Add 1 mL of reverse osmosis (RO) water to the tube and resuspend the beads using a vortex mixer. Place the tube into a magnet, allow magnetic separation, then aspirate the supernatant (washing step). Repeat this step twice with water. **Tip:** a centrifuge can be used to remove beads from the lid of the tube after vortex mixing (<5 seconds at 1,500 rpm).
4. Repeat the washing step using 1 mL of 0.1 M MES pH 5.0 buffer.
5. Remove the beads from the magnet and add 200 µL of 0.1 M MES pH 5.0 buffer.
6. Add 200 µL of a 3 mg/mL protein solution (60 µg/mg at saturation condition). Gently mix the beads into a homogeneous solution with a vortex mixer. For optimal performance, a titration of the amount of protein is recommended.
7. Place the tubes on the bottle roller to mix the beads at room temperature for 30 minutes.
8. Prepare the EDC activation solution: Dissolve 10 mg of EDC (N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride >98% Purum; mol. wt. 191.7 g/mol) in 3.5 mL of 0.1 M MES pH 5.0 buffer for a stock solution of 15 mM.
   - **Important:** EDC is hygroscopic and can undergo rapid hydrolysis in aqueous solutions. For optimum results, the EDC activation solution must be prepared right before addition to the samples, and it remains active within 15 minutes after preparation.
9. Add 100 µL of the EDC activation solution to the 400 µL bead and protein dispersion. Vortex the tubes for 10 seconds and place them on the bottle roller for 1 hour at room temperature. **Note:** Do not prepare the EDC solution more than 5 minutes before adding it to the beads.
   - When 100 µL is added to 200 µL of protein solution and 200 µL of beads in MES, this results in a 3.0 mM solution. This is the recommended concentration to use as a starting point, but a wider EDC concentration range can be used as part of optimization.
10. Wash the coupled beads as in step 3 – once with 1.5 mL of PBST buffer, and twice with 1.5 mL PBS buffer.
11. Resuspend the beads with a desired buffer and roll the tubes for a minimum of 1 hour before any follow-up applications. Store at 2 to 8 °C. Do not freeze.

Characterization techniques for Agilent magnetic beads

BCA test

The bicinchoninic acid (BCA) assay is a colorimetric assay that can be used to measure the total protein concentration successfully coupled to magnetic beads (nonspecifically bound and supernatant protein can contribute to result).

This assay relies on two chemical reactions. The process starts when the peptide bonds of the bound protein reduce the Cu^{2+} ions from the copper (II) sulfate (reagent B) to Cu^{+}. This ion will then chelate with two molecules of BCA, resulting in a purple-colored complex that absorbs light at 562 nm wavelength, and is directly proportional to protein concentration.

Material and calculations required to perform the assay

- Pierce BCA Protein Assay kit (reagent A and B)
- 500 µL of protein prepared in PBS buffer at 2 mg/mL
- Water bath for carrying binding capacity characterization assay
- 96-well clear-bottom black microplate
- Microplate reader capable of reading absorbance at 562 nm

To calculate the amount of reagent A and reagent B needed in mL, the following formulas should be applied:

a. **Reagent A (mL):** (number of samples + number of standards) × 4.2

b. **Reagent B (mL):** amount of reagent A/50

Assay protocol

1. Roll the recently coupled beads for at least 30 minutes before starting with this assay and prepare a water bath at 37 °C.
2. Prepare 500 µL of 2 mg/mL protein solution in the PBS buffer. This solution will be used to prepare the calibration standards:

Standard Concentration (ng/mL)	PBS Buffer Volume (µL)	Protein Solution Volume (µL)
0	250	0
200	225	25
400	200	50
600	175	75
800	150	100

3. In a Nalgene bottle, mix reagent A and reagent B to obtain a green solution and aliquot 4 mL in different 10 mL tubes.
4. Add 200 µL of the standards and samples in different 10 mL tubes. Mix the samples and standards with the green solution using a vortex mixer and place the tubes in the water bath for 30 minutes.
5. Cool down the tubes for 1 minute by submerging them in cold water to slow the color change, and place the tubes in a magnet for magnetic separation.
6. Pipette 300 µL of the colored solution into the microplate in triplicate and perform an absorbance reading at 562 nm wavelength. **Note:** Pipetting should be carried out with the minimum delay of time for an accurate reading of the binding capacity, as color will still develop with time.
7. Plot the calibration curve and calculate binding capacity using the slope, the intercept, and the dilutions carried out in binding protocol (50 mg of beads in 7.5 mL).

Choose your options to achieve quality characterization

Technical information

Specifications	LodeStars Beads	LodeStars High Bind Beads
Diameter	2.7 µm	2.7 µm
Iron Content	~ 20%	~ 20%
Carboxyl Binding Capabilities		
BCA assay*	~20 µg/mg bead	~40 µg/mg bead

* Assay details available on request.

Ordering information

Description	LodeStars beads (30 mg/mL)		LodeStars High Bind beads (50 mg/mL)	
LodeStars Carboxyl beads	2 mL	PL6727-0001	1 mL	PL6827-0001
	10 mL	PL6727-0003	10 mL	PL6827-0003
	100 mL	PL6727-0005	100 mL	PL6827-0005
	400 mL	PL6727-0006	400 mL	PL6827-0006
	800 mL	PL6727-0007		

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