Protocol

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Coupling Protocol for LodeStars Streptavidin Magnetic Beads

Introduction

The following protocol provides users with guidance for the successful coupling of biotinylated biomolecules to Agilent LodeStars Streptavidin magnetic beads. As an example of a fluorescent molecule, biotin-4-fluorescein was selected for biotin-specific quantification purposes. Different biotinylated molecules will require further optimization to achieve maximum performance in your application.

Biotin coupling protocol

Materials and reagents

- 2,000 to 2,500 nmol/mL Biotin-4-fluorescein stock solution (B4F, 644.69 g/mol) in 0.1 M sodium bicarbonate, pH 9.0
  - Alternate pH conditions can be explored as part of optimization
- PBS-BSA buffer: PBS 0.1 M sodium phosphate, pH 7.4, with 0.1% w/v BSA, 0.1% w/v Tween 20, 0.05% w/v sodium azide
- 0.1 M NaOH 0.1% w/v SDS solution: 0.1 M sodium hydroxide (NaOH), 0.1% w/v sodium dodecyl sulphate (SDS)
- 2 mL Microcentrifuge tubes
- Centrifuge tubes
- Magnetic separator rack
- Microcentrifuge
- Vortex mixer
- Drying oven
- Bottle roller
- White microplate
- Spectrophotometer with fluorescence capability

Before starting the binding protocol

- Check the expiration date of the buffers (buffers should have been prepared less than 6 months ago).
- Allow buffers and reagents to reach room temperature. **Note:** If frozen, defrost the B4F solution in the dark 30 minutes before use.
- Disperse the LodeStars Streptavidin beads using a bottle roller for a minimum of 2 hours for volumes above 250 mL, or 1 hour for smaller volumes. Nonhomogeneous dispersion could result in an incorrect quantity of beads being used in conjugation or aggregations, causing incomplete coupling. For optimal bead dispersion, **Agilent recommends rolling beads at room temperature overnight (~16 hours)**.
- For binding capacity studies, where the biotinylated protein needs to be eluted, preheat the drying oven to 80 °C.

Binding procedure

1. Aliquot 1 mg of LodeStars Streptavidin beads (~33 µL) or LodeStars High Bind Streptavidin beads (~20 µL) into a 2 mL microcentrifuge tube. **Note:** The exact volume needed for 1 mg can be calculated using the certificate of analysis.
2. Place the tube in a magnetic separator rack to separate the beads from the solution. Once complete, aspirate the supernatant and remove the tube from the magnet.
3. Add 1 mL of PBS-BSA buffer (0.1 M sodium phosphate, pH 7.4, with 0.1% w/v BSA, 0.1% w/v Tween 20, PBS pH 7.4, 0.05% w/v sodium azide) to the tube and resuspend the beads using a vortex mixer. Place the tube into a magnetic separator rack, allow magnetic separation, and then aspirate the supernatant (washing step). Repeat this step twice. **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. For a B4F molecule coupling, prepare 20 nmol/mL of solution in PBS-BSA buffer. For other biotinylated conjugates, a titration of the coupling concentration is recommended to determine optimal performance.
5. Remove the beads from the magnetic separator rack and add 700 µL of the biotinylated solution. Gently mix the beads into a homogeneous solution with a vortex mixer. Place the tubes in a dark bottle to prevent UV exposure.
6. Place the dark bottle on the bottle roller to mix the beads at room temperature for a minimum of 10 minutes.
7. Place the tube in a magnetic separator rack. Once the separation is complete, aspirate the supernatant then remove the tube from the magnetic separator rack. Wash the coupled beads (as in step 3) three times with 1.5 mL of PBS-BSA buffer.
8. 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

Protein eluting procedure

1. After successfully coupling biotinylated molecules to LodeStars Streptavidin beads, place the tube in a magnetic separator rack to separate the beads from the solution. Once the separation has been completed, aspirate the supernatant then remove the tube from the magnet.
2. Add 1.5 mL of 0.1 M NaOH 0.1% w/v SDS solution. Gently mix the beads into a homogeneous solution with a vortex mixer and place the tubes in an upright position in the oven at 80 °C for 180 minutes. **Tip:** A centrifuge can be used to remove beads from the lid of the tube after vortex mixing (<5 seconds at 1,500 rpm).

Note: Heating will denature the protein impacting structure and function.

For binding capacity measurements, the 0.1 M NaOH 0.1% w/v SDS buffer is the most effective buffer for complete SA-biotin cleavage. However, for biomolecules, these conditions may not be suitable. Recoveries of 50% were obtained by replacing 0.1 M NaOH 0.1% w/v SDS buffer by the following buffers and conditions:

- 0.1% v/v Formic acid, 70% v/v acetonitrile: roll the beads for 60 minutes at 37 °C
- 0.1% v/v Formic acid, 30% v/v acetonitrile: roll the beads for 30 minutes at 37 °C

Quantification of fluorescence molecules

1. Remove the samples from the oven, place the tubes in a magnetic separator rack, and allow them to reach room temperature in the dark.
2. From the biotinylated solution, prepare a 640 pmol/mL solution in the elution buffer. Use this solution to prepare the calibration standards:

Standard Concentration (pmol/mL)	Elution Buffer Volume (µL)	640 pmol/mL Solution Volume (µL)
0	1,000	0
20	969	31
40	938	62
80	875	125
160	750	250
320	500	500

Note: Use a 640 nmol/mL stock solution to adjust gain settings on the fluorimeter.

3. In a new centrifuge tube, mix 900 µL of the buffer used to elute the samples and 100 µL of sample supernatant. **Note:** This dilution does not apply to standards.
4. Pipette 100 µL of standards and diluted samples into the microplate in triplicate and perform fluorescence reading.
5. Plot the calibration curve and calculate the binding capacity using the slope, the intercept, and the dilutions carried out in the binding protocol (dilution factor of 15).

Choose your options to achieve quality characterization

Ordering information

	LodeStars Beads (10 mg/mL)		LodeStars High Bind Beads (50 mg/mL)	
LodeStars Streptavidin Beads	2 mL	PL6727-1001	1 mL	PL6827-1001
	10 mL	PL6727-1003	10 mL	PL6827-1003
	100 mL	PL6727-1005	100 mL	PL6827-1005
	800 mL	PL6727-1007	400 mL	PL6827-1006

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