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Dynabeads™ Cell Separation V.1

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Protocol status: In development
We are still developing and optimizing this protocol. If you use it and find any problems or different ways to obtain better results, let us know!

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Abstract

The aim of this protocol is to separate the aptamers that bind to the cells from the ones that don't. The cells used have a histidin tag that attaches to the beads' streptavidin tag. The beads will then pull the cells and the complex will precipitate. Since the aptamers have high specifity and affinity for particular sequences, those able to recognice the cells will bind to them, being part of the complex. The aptamers that don't recognice the cells, won't bind. This protocol enables to separate aptamers based on their affinity using the target cells.

The Dynabeads His-Tag Isolation and Pulldown from ThermoFisher is an already proven system to separate and isolate proteins. We have automatised the system with the Opentrons to separate cells and it worked succesfully. But if you don't have an Opentrons machine, it is also possible to perform a manual protocol as described here.

Guidelines

Calculations

- Co = initial
- Cb = bound
- Cnb = non bound
- Cf = final
- Cne = non eluted

$Cb = Co - Cnb$
$Cne = Cb - Cf$
$Cnb \text{ total} = Cnb + Cw1 + Cw2$

The procedures indicate to apply the tube to the magnet for 2 min, but the sample can be handled when the beads are visually observed to be collected at the tube wall and the liquid is clear. This is supported by manufacturer.

Materials

MATERIALS

Dynabeads™ His-Tag Isolation and Pulldown **Thermo Fisher** **Catalog #**10103D

Laboratory equipment:

- Magnetic rack
- Micropipettes (10-1000 µL)
- Autoclave
- Incubator
- Culture plates
- Eppendorf tubes / microcentrifuge tubes
- Spectrophotometer cuvettes
- Spectrophotometer
- Vortex
- Balance shaker

Reagents:

- PBS
- Dynabeads™ Protein Purification System (as indicated above)
- Wash/Binding Buffer 2x: 100mM Sodium-Phosphate (pH 8.0) + 600 mM NaCl + 0.02% Tween-20.
- His Elution Buffer: 300mM Imidazole + 50 mM Sodium-phosphate (pH 8.0) + 300 mM NaCl + 0.01% Tween-20.
- Pull-down Buffer 2x: 6,5 mM Sodium-phosphate (pH 7.4) + 140mM NaCl + 0.02% Tween-20.
- Buffer modifiers: 1M NaCl, 0.1M Imidazole
- Agar
- *E.coli* + His (cells)
- *E.coli* control ⊖ (cells)

Reagents	
Description	**Amount**
E. coli + His (cells)	800 µL (Binding Buffer), 10^6 CFU/mL
E. coli control ⊖ (cells)	800 µL (Binding Buffer), 10^6 CFU/mL
Dynabeads™ His	200 µL
Washing buffer (x2)	1,2 mL
Elution buffer	200 µL
Washing buffer	200 µL
Sample Co	300 µL Washing buffer + 50 µL cell sample
Sample Cnb	300 µL Washing buffer + 50 µL supernatant
Sample Cw	300 µL Washing buffer + 50 µL supernatant
Sample Cf	270 µL Washing buffer + 30 µL Elution buffer + 50 µL supernatant
Sample Cne	300 µL Washing buffer + 50 µL beads in LB
Blank Co, Cnu, Cw	350 µL Washing buffer
Blank Cf	315 µL Washing + 35 µL Elution buffer

Table 1: note than, when "supernatant" is indicated, each time refers to a different supernatant according to steps. Be careful to label the tubes correctly and not mix different supernatants in the same tube.

Safety warnings

Lab coat and gloves should be worn throughout the whole experiment. All working surfaces must be clean and all the reactives should be treated following manufacturer's instructions.

Before start

- Prepare the sample containing the histidine-tagged cells in a total volume of 700 µL, 1X Binding/Wash Buffer, 10^6 UFC/mL.
- Prepare a similar sample of regular cells, to be used as negative control.
- The following steps need to be applied to both samples similarly.
- Set the thermal module at 4°C.
- Wash/Binding Buffer 2x and pull-down Buffer 2x must be diluted in proportion 1:1 before use.

1 Thoroughly resuspend the Dynabeads™ magnetic beads in the vial using a pipette or a vortex. This must be done each time the beads are going to be used.

2 Transfer 100 µL (4 mg) Dynabeads™ to a 1.5 mL eppendorf tube. Place the tube in the magnetic rack for 2 min, to ensure separation. Then aspirate and discard the supernatant.

Add your cell sample (Sample $\mathbf{C_o}$, prepared in 1X Binding/Wash Buffer) to the beads. Mix well.

3 Incubate for 5 min at room temperature in a balance shaker (moving gently). The incubation time may be increased up to 10 min.

4 Place the tube on the magnet rack for 2 min, then remove the supernatant and keep it in a new tube (Sample $\mathbf{C_{nb}}$).

5 Wash the beads 3 times with 400 µL 1X Binding/Wash Buffer by placing the tube on the magnet for 2 min and remove the supernatant to a new tube.
Resuspend the beads thoroughly between each washing step. The supernatant is kept in the same tube for every wash (Sample $\mathbf{C_w}$).

6 Add 100 µL His-Elution Buffer. Incubate the suspension for 5 min at room temperature in the balance shaker (moving gently).

7 Apply on the magnet for 2 min and transfer the supernatant containing the eluted histidine-tagged cell to a clean tube (Sample $\mathbf{C_f}$).

8 Resuspend the remaining beads in 100 µL LB and transfer it to a new tube (Sample $\mathbf{C_{ne}}$). Proceed to cell counting:

8.1 a. Make serial dilutions of each of the samples until a concentration of 10^{-4} in LB. Plate 100 µL of the each final dilution in LB agar + kanamicine plates.

b. Count the cells by using a OD relationship.