Dynabeads™ Cell Separation V.1

Manuela De Las Casas, Laura Sánchez

1Universidad Complutense de Madrid, AEGIS - Madrid iGEM 2019

AEGIS - Madrid iGEM 2019

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 specificity and affinity for particular sequences, those able to recognize the cells will bind to them, being part of the complex. The aptamers that don't recognize 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 successfully. But if you don't have an Opentrons machine, it is also possible to perform a manual protocol as described here.

DOI:
dx.doi.org/10.17504/protocols.io.8h6ht9e

Protocol Citation: Manuela De Las Casas, Laura Sánchez 2019. Dynabeads™ Cell Separation. protocols.io https://dx.doi.org/10.17504/protocols.io.8h6ht9e

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Calculations

- \( C_b = C_i - C_n_b \)
- \( C_{ne} = C_b - C_f \)
- \( C_{nb}\) total = \( C_n_b + C_{w1} + C_{w2} \)

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**

- **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
- \textit{E.coli} + His (cells)
- \textit{E.coli} control \oplus (cells)

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. coli} + His (cells)</td>
<td>800 (\mu)L (Binding Buffer), 10(^6) CFU/mL</td>
<td></td>
</tr>
<tr>
<td>\textit{E. coli} control \oplus (cells)</td>
<td>800 (\mu)L (Binding Buffer), 10(^6) CFU/mL</td>
<td></td>
</tr>
<tr>
<td>Dynabeads\textsuperscript{TM} His</td>
<td>200 (\mu)L</td>
<td></td>
</tr>
<tr>
<td>Washing buffer (x2)</td>
<td>1,2 mL</td>
<td></td>
</tr>
<tr>
<td>Elution buffer</td>
<td>200 (\mu)L</td>
<td></td>
</tr>
<tr>
<td>Washing buffer</td>
<td>200 (\mu)L</td>
<td></td>
</tr>
<tr>
<td>Sample Co</td>
<td>300 (\mu)L Washing buffer + 50 (\mu)L cell sample</td>
<td></td>
</tr>
<tr>
<td>Sample Cnb</td>
<td>300 (\mu)L Washing buffer + 50 (\mu)L supernatant</td>
<td></td>
</tr>
<tr>
<td>Sample Cw</td>
<td>300 (\mu)L Washing buffer + 50 (\mu)L supernatant</td>
<td></td>
</tr>
<tr>
<td>Sample Cf</td>
<td>270 (\mu)L Washing buffer + 30 (\mu)L Elution buffer + 50 (\mu)L supernatant</td>
<td></td>
</tr>
<tr>
<td>Sample Cne</td>
<td>300 (\mu)L Washing buffer + 50 (\mu)L beads in LB</td>
<td></td>
</tr>
<tr>
<td>Blank Co, Cnu, Cw</td>
<td>350 (\mu)L Washing buffer</td>
<td></td>
</tr>
<tr>
<td>Blank Cf</td>
<td>315 (\mu)L Washing + 35 (\mu)L Elution buffer</td>
<td></td>
</tr>
</tbody>
</table>

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 INSTRUCTIONS

- Prepare the sample containing the histidine-tagged cells in a total volume of 700 μL, 1X Binding/Wash Buffer, 10⁶ 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 \( C_o \), 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 \( C_{nb} \)).

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 \( C_w \)).

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 \( C_f \)).
Resuspend the remaining beads in 100 μL LB and transfer it to a new tube (Sample C_\text{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.