Magnetic Beads Cell Separation V.1
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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 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 magnetic beads from Promega have proven to be an efficient method to separate proteins. With this variation, we aimed to do a proof of concept and test if we could separate cells efficiently as well. Unfortunately, the method didn't work for us and we weren't able to achieve a good result. We still want to optimize this process, but the protocol that we already developed might help other scientists.
GUIDELINES

Calculations
- \( C_0 \) = initial
- \( C_b \) = bound
- \( C_{nb} \) = non bound
- \( C_f \) = final
- \( C_{ne} \) = non eluted

\[ Cu = C_0 - C_{nb} \]
\[ C_{ne} = Cu - C_f \]
\[ C_{nu \: total} = C_{nu} + C_w1 + C_w2 \]

Optimization
Several tests must be performed following the protocol with different values for each parameter, as shown in the next table. The aim is to find the minimum values which result in an acceptable efficiency.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>pLB89</th>
<th>pLH1</th>
<th>pLH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (UFC/mL)</td>
<td>10^4</td>
<td>10^4</td>
<td>10^4</td>
</tr>
<tr>
<td>Incubation time (min)</td>
<td>30 45 60</td>
<td>30 45 60</td>
<td>30 45 60</td>
</tr>
<tr>
<td>Dilutions plated</td>
<td>10^7 10^7 10^7</td>
<td>10^7 10^7 10^7</td>
<td>10^7 10^7 10^7</td>
</tr>
</tbody>
</table>

TOTAL: 81 plates
MATERIALS

High Capacity Magne™ Streptavidin Beads Promega Catalog #V7820

Laboratory equipment:
- Magnetic rack
- Micropipettes (10-1000 μL)
- Autoclave
- Incubator
- Culture plates
- Eppendorf tubes / microcentrifuge tubes

Reagents:
- PBS
- MagneHis™ Protein Purification System (as indicated above)
- Wash Buffer: HEPES + Imidazole 10%.
- Elution Buffer: HEPES + Imidazole 500 mM.
- Agar

SAFETY WARNINGS

- Lab coat and disposable gloves are recommended throughout the whole procedure.

BEFORE START INSTRUCTIONS

Be sure to have enough amount of the buffers as well as the other reagents.

Separation with the MagneHis+

1. Prepare the magnetic module: remove the magnetic plate and load the necessary number of 1.5-mL microcentrifuge tubes.

2. Prepare fresh cultured E. coli samples with cell concentrations of $10^6$ CFU/mL in PBS:

2.1. Measure the optical density of the bacterial inoculum at 600 nm absorbance (OD 600)
2.2 Dilute it until you get the correct concentrations based on the relation: 1 OD < >1E9 CFU/mL [1].

**Collect sample C₀**

3 Add 1 mL of each sample [2] to a new eppendorf. Add 300 μL of MagneHis™ [3] to the bacteria mixture. Make sure cells are resuspended properly by gentle mixing.

4 Incubate at room temperature for 30, 45 and 60 min each under gentle continuous agitation to prevent the beads from settling.

5 Insert the magnetic plate into the magnetic module. Allow the tube to stand for 3 min for maximum recovery.

6 Remove the supernatant with the pipette, very carefully, and put it in a new tube.

**Collect sample Cₙb**

7 Add 1 mL of Wash Buffer (or PBS) and resuspend by inversion. Allow the tube to stand for 3 min into the magnetic module.

8 Repeat the washing process (steps 6-7) twice.

**Collect sample Cₚ₁, Cₚ₂**

9 Resuspend the MagneHis™ complex in 100 μL of Elution Buffer. Mix briefly by inversion. Allow the tube to stand for 1’5 min into the magnetic module.
Take the supernatant with the pipette carefully and add it to a 900 μL LB eppendorf.

* Imidazole is very aggressive to the cell membrane, but it is needed to break the interaction between histidine and MagneHis™, therefore a high dilution is performed to stop its effect.

Collect sample $C_f$

Resuspend the beads in 100 μL LB.

Collect sample $C_{ne}$

For spread plating, a 10-fold dilution series (until dilution 10^{-7}) is prepared. Plate 100 μL from dilutions 10^{-1}, 10^{-2} and 10^{-3} onto 3 different agar plates with the pertinent antibiotic. The plates are incubated at 37°C for 48 h. The colonies are numerated, and the result is expressed as CFU/mL [4].

We have developed this protocol from reading the following bibliography:


