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Magnetic Beads Cell Separation V.1

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Protocol status: In development
We are still developing and optimizing this protocol. If you try it and find a better way to perform it, 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 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_o = initial
- C_b = bound
- C_{nb} = non bound
- C_f = final
- C_{ne} = non eluted

$$Cu = Co - Cnu$$
$$Cne = Cu - Cf$$
$$Cnu\ total = Cnu + Cw1 + Cw2$$

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.

Cell line	pLBB9			pLH1			pLH2		
Concentration (UFC/mL)	10^1	10^3	10^6	10^1	10^3	10^6	10^1	10^3	10^6
Incubation time (min)	30 45 60	30 45 60	30 45 60	30 45 60	30 45 60	30 45 60	30 45 60	30 45 60	30 45 60
Dilutions plated	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}	10^{-6} 10^{-7} 10^{-8}

TOTAL: 81 plates

Materials

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

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 C_o

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 C_{nb}

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 C_{w1}, C_{w2}

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.

10 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

11 Resuspend the beads in 100 µL LB.

Collect sample C_{ne}

12 For spread plating, a 10-fold dilution series (until dilution 10-7) is prepared. Plate 100 µLfrom 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].

REFERENCES

13 We have developed this protocol from reading the following bibliography:

[1] Park J, Gasparrini A, Reck M, Symister C, Elliott J, Vogel J et al. Plasticity, dynamics, and inhibition of emerging tetracycline resistance enzymes. Nature Chemical Biology. 2017;13(7):730-736. doi: 10.1038/nchembio.2376

[2] Lim M, Lee G, Huynh D, Hong C, Park S, Jung J et al. Biological preparation of highly effective immunomagnetic beads for the separation, concentration, and detection of pathogenic bacteria in milk. Colloids and Surfaces B: Biointerfaces. 2016;145:854-861.

[3] http://t-takaya.net/manual/Miltenyi_Biotec_130-090-485.pdf.

[4] Walcher G, Stessl B, Wagner M, Eichenseher F, Loessner M, Hein I. Evaluation of Paramagnetic Beads Coated with Recombinant Listeria Phage Endolysin–Derived Cell-Wall-Binding Domain Proteins for Separation of Listeria monocytogenes from Raw Milk in Combination with Culture-Based and Real-Time Polymerase Chain Reaction–Based Quantification. Foodborne Pathogens and Disease. 2010;7(9):1019-1024. doi: 10.1089/fpd.2009.0475