



Oct 20, 2019

# Magnetic Beads Cell Separation V.1

Laura Sánchez<sup>1</sup>

<sup>1</sup>AEGIS - Madrid iGEM 2019

In Development

dx.doi.org/10.17504/protocols.io.8gvhtw6

AEGIS - Madrid iGEM 2019



Manuela de las Casas

Universidad Complutense de Madrid, 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 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

$$C_u = C_o - C_{nb}$$

$$C_{ne} = C_u - C_f$$

$$C_{nu\ total} = C_{nu} + C_{w1} + C_{w2}$$

### 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.

Cell line	pLBB9			pLH1			pLH2		
Concentration (UFC/mL)	10 <sup>1</sup>	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>1</sup>	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>1</sup>	10 <sup>3</sup>	10 <sup>6</sup>
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 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-6</sup>
	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-7</sup>
	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>	10 <sup>-8</sup>

TOTAL: 81 plates

## MATERIALS

NAME 	CATALOG # 	VENDOR 
High Capacity Magne™ Streptavidin Beads	V7820	Promega

## MATERIALS TEXT

### 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 STARTING

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:  $1 \text{ OD} < > 1 \text{E9 CFU/mL}$  [1].

### Collect sample $C_0$

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<sub>nb</sub>**

- 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<sub>w1</sub>, C<sub>w2</sub>**

- 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<sub>f</sub>**

- 11 Resuspend the beads in 100 µL LB.

**Collect sample C<sub>ne</sub>**

- 12 For spread plating, a 10-fold dilution series (until dilution 10<sup>-7</sup>) is prepared. Plate 100 µL from dilutions 10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup> onto 3 different agar plates with the pertinent antibiotic. The plates are incubated at 37°C for 48 h. The colonies are enumerated, 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](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



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited