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Streptavidin-CBD purification

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Protocol status: In development
We are still developing and optimizing this protocol. It is possible that when we optimize it, several changes occur. If you find a way to make it work better, let us know!

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Abstract

The aim of this protocol is to obtain the purified streptavidin with cellulose binding domains on both sides from the transformed bacteria *E. coli*. Later on, this protein will be attached to a membrane as part of the detection system.
The obtention of the proteins starts at this point with transformed bacteria (*E. coli*).
The previous steps to obtain the transformed bacteria and further steps to use the purified protein do not correspond to this protocol.

Materials

MATERIALS

- 15 ml sterile falcon tubes and rack
- 1.5 mL Eppendorf tubes
- 37°C Incubator
- Glucose
- Shaker incubator
- Sterile conditions (e.g. laminar flow or a flame)
- Centrifuge
- Microcentrifuge
- Liquid LB medium
- Ultrospec 1100 pro
- Disposable Cuvettes **Thermo Fisher Catalog #**14955125
- Reusable Glass Narrow-Mouth Erlenmeyer Flasks, 125mL **Thermo Fisher Catalog #**FB500125

- Pipettes
- Filter paper
- Permanent pen
- Digralsky planting handle

Safety warnings

All the protocols involving bacteria should be performed with gloves, lab coat and aseptic conditions (laminar flow cabinet or near Bunsen's lighter flame). Although the cells are not dangerous, all contaminations should be avoided.

Cells should never be disposed in the sink and sterile material (autoclaved/disposable) is highly recommended. Working surfaces should be washed with alcohol before starting to work as well as after finishing.

Before start

Make sure you have transformed bacteria by checking the incorporation of the plasmid (this can be done by adding chloranphenicol to the culture). It is also possible to check if the streptavidin insert is incorporated. To ensure, digest the DNA with NotI (which will release the recombinant fragment) and run it in a gel.

Growing the cells

1 From the Petri dishes with the bacteria, pick a few colonies with a Digralsky planting handle and resuspend the colonies in liquid LB in an Erlenmeyer flask. It is recommended to set more than one flask, to minimize risks in case one of the flasks goes wrong. Grow a negative control as well (the bacteria without the plasmid).

1.1 Let the bacteria grow in an incubator at 37ºC until it reaches an OD600 of 0.6 approximately (range 0.4-0.8). Check periodically the OD with the ultrospec. To check the OD, set a blank cuvette with just LB to compare the bacteria grow.
Be careful not to overgrow the bacteria. If overgrown, they won't be useful for transformation. The culture should be at log phase.

The volume pipetted into the cuvettes is discarded after the measurements. Do not return it to the flask.

Induction of the expression

2 Since the promoter used for this bacteria has a constitutive expression, there is no need to induce. But depending on the promoter you used, you might need to induce the expression of the protein.

In our case, we just need to let the bacteria grow as step 1.1 indicates.

Purification of the protein

3 To purifiy the protein, it is necessary to lysate the cells. In order to do so:

3.1 Pick 1-2 mL of bacteria, centrifugate 3-5 min at 15000 x g. After centrifugation, take out the supernatant carefully and resuspend the cells pellet with B-mercaptoethanol and SDS.

Comprobation of the procedure

4 OPTIONAL. This step is not necessary to obtain the protein. But with the purified protein, it is recommended to check that the procedure has gone right and that we have the desired protein.

Boil the samples 3-5 min at 98ºC-100ºC. After boiling, centrifugate again for 3-5 min at 15000 x g.

Run an electrophoresis (follow an electrophoresis protocol). Recommended to use 1:10 and 1:100 dilutions from the supernatant in an SDS-page acrylamide gel (pore size 10-12%).

Materials and steps for the electrophoresis are not contemplated in this protocol.