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Working

## Protein Expression

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### ABSTRACT

The protein expression is a process about synthesizing and modification for proteins in living organisms with using several laboratory techniques and devices. This technique is generally used for obtaining protein from the competent cell that is used for carrying plasmids that possess the protein that we want to express.

### Protein expression involves two general steps that;

- Gene expression; which is continuum that converting DNA to the mRNA that can be translate into protein.
- Protein manufacturing; which is translation process.

### STEPS MATERIALS

| NAME     | CATALOG # | VENDOR  | CAS NUMBER | RRID |
|----------|-----------|---------|------------|------|
| LB Broth | J106-2KG  | Amresco |            |      |

Transform BL21 cells and plate them on Amp (Kan).

1

Afer overnight incubation, take colonies and transfer into 6 ml LB with 6 µl Amp. Let them grow for 3-5 hours.

2 37 °C



LB Broth  
by Amresco  
Catalog #: J106-2KG

6 ml LB

6 µl Ampicillin

When incubation process is done, transfer it to 1 L ZYM-5052 Medium with 1 µl Amp.

3

Centrifuge at 6000 rpm for 10 minutes.

4



SORVALL LYNX 4000  
Centrifuge  
Thermo Scientific 75006580

⌚ 00:10:00 Centrifuge 🌡 15 °C

Discard liquid and repeat centrifuge step for 3 times total.

5 ⌚ 00:10:00 2nd Centrifuge

⌚ 00:10:00 3rd Centrifuge

Add Binding Buffer.

6 🗑 20 ml Binding Buffer

Transfer it to 50 ml falcon tube.

7 In this step 35 µl of total sample should be obtained. Binding Buffer involves 5 ml Imidazole, 20 ml TBS, ddw up to 200 ml.

For the resuspension of pallet, tubes with Binding Buffer can be put into shaker.

8

Sonication will be occurred.

9 For sonication, plastic test tubes are usually used for sonication. This sonication process is done at 3 cycles for 7 minutes.

⌚ 00:07:00 Sonication for 1 cycle.

Repeat this step for 3 times total.

Centrifuge at 13000 rpm for 30 minutes.

10 ⌚ 00:30:00 Centrifuge 🌡 6 °C



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