May 07, 2019

Protein Expression

DOI
dx.doi.org/10.17504/protocols.io.sj2ecqe

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Protocol Citation: Rengin Yamur Akbiyik, Sevval Uysalcan 2019. Protein Expression. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.sj2ecqe**

Protocol status: Working
We use this protocol and it's working

Created: August 13, 2018

Last Modified: May 07, 2019

Protocol Integer ID: 14682

Keywords: Protein Expression

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.

Materials

STEP MATERIALS

LB Broth **Amresco Catalog #**J106-2KG

Protocol materials

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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 **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

Equipment	
SORVALL LYNX 4000	NAME
Centrifuge	TYPE
Thermo Scientific	BRAND
75006580	SKU

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 occured.

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