

# Inoculation of the LB medium, expression of the protein and harvesting of the bacterial culture

The inoculation of the LB medium with the transformed bacterial strain, the subsequent expression of the protein and the harvesting of the bacterial culture are the first steps to protein purification and are essential for successful results.

## Materials

LB Medium  
Antibiotics  
Bacteria solution  
Cuvette  
Falcon

## Instruments:

Centrifuge  
Shakers  
Photometer

## Procedure

Pour LB media into piston (1.8L each)  
Add 1.8ml (1/1000) antibiotics and 20ml bacteria solution  
Shake at 37°C 100 rpm

Take samples (measure OD hourly)  
At an OD of 0.6 add 730µl IPTG  
Set shaker to 17°C 100rpm  
Shake culture overnight at 17°C 100 rpm.

The next day bacteria solution centrifugation 7k 12min  
Dry pellet and transfer to Falcon  
Determine the weight of the wet mass  
Store or lyse bacteria pellets at -80°C

## Trouble shooting

Overgrowth of the bacteria should be avoided before induction (<0.6).  
The antibiotic should be added fresh.