

Comparison of protein expression in different strains

The EnBase™-48DWP is an excellent tool in which you perform parallel cell cultivation and protein expression. Optimise strain, media, buffers and additives in a single plate. Shake flask protein yields in a high throughput 48DWP format. Achieve more with EnBase.

Here we demonstrate the application of EnBase™-48DWP to compare the expression level of the his-tagged protein TrpF in six different *E. coli* strains with subsequent purification using Ni-NTA columns. The cultivation of each strain was performed in 4 parallels resulting in a total cultivation volume of 6 ml per expression system.

Recombinant
Production of
Protein



Protocol from cloning to purification

Transformation of the pET construct into six *E. coli* strains

Harvest of colonies from transformation plate by resuspension in medium and measurement of OD₆₀₀

Inoculation of EnBase™ 48DWP and cultivation at 37°C

IPTG induction after 22h, shift to 20°C

Harvest after 40 h cultivation time

Cell lysis

Affinity purification by using Ni-NTA (250 µl columns)

Detection of protein concentration by absorbance measurement

EnBase™-48DWP cultivation

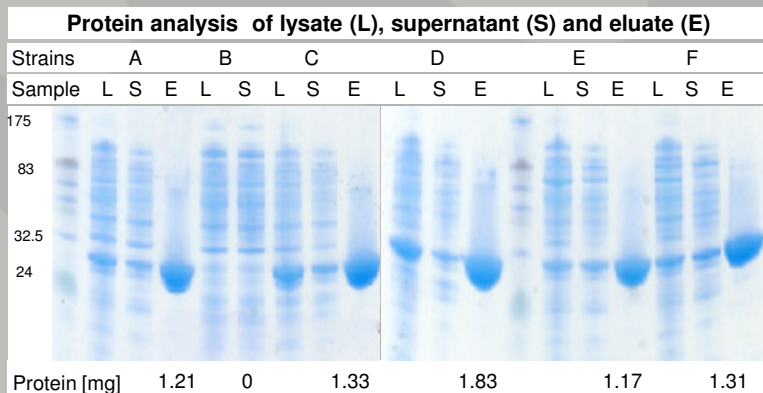
E. coli strains:

- A = BL21 Star (DE3)
- B = BL21 Star (DE3) pLysS
- C = BL21 (DE3) pRARE2
- D = Rosetta2 (DE3) pLysS
- E = BL21 (DE3) cc3 (GroE)
- F = BL21 (DE3) cc4 (GroE, DnaK, ClpB)

Start OD₆₀₀ = 0.1

Induction OD₆₀₀ = 8.9 - 15.9

Final OD₆₀₀ = 14.0 - 50.2



Highest yield was obtained with *E. coli* Rosetta2 (DE3)pLysS: 1.83 mg purified protein from 4 EnBase™-48DWP wells corresponding to a volumetric yield of 305 mg / L.