

Express-timator™ Kit



Cat. No. R1001K

For more detailed information please refer to the Semba Biosciences website www.sembabio.com.

DESCRIPTION

The Express-timator Kit is used to estimate target recombinant protein expression levels in *E. coli* cell lysates. The kit contains a set of standards consisting of soluble BL21 cell lysate containing known amounts of a 52.2-kDa recombinant protein. The standards are run along-side cell lysate samples containing expressed target proteins on an SDS-polyacrylamide gel followed by Coomassie blue staining. By comparing band intensities vs. the known standards, the target protein level can be estimated fairly accurately. It is important to have a reasonable estimate of the target protein concentration for maximum performance of many chromatographic procedures, including purification with the Octave™ Chromatography System.

KIT COMPONENTS

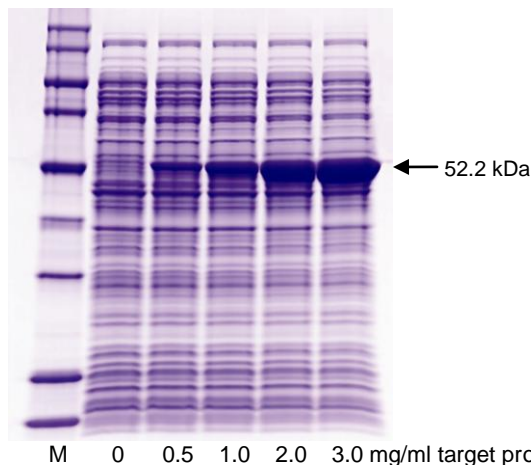
- # R1020E Express-timator Standard 0 mg/ml
- # R1021E Express-timator Standard 0.5 mg/ml
- # R1022E Express-timator Standard 1.0 mg/ml
- # R1023E Express-timator Standard 2.0 mg/ml
- # R1024E Express-timator Standard 3.0 mg/ml
- # R1025E SDS Sample Buffer, 4X

PROTOCOL

1. Prepare the soluble cell lysate* and measure total protein concentration by the bicinchoninic acid (BCA) method or another protein assay.
2. Mix 25 μ l lysate with 50 μ l deionized water and 25 μ l SDS Sample Buffer, 4X.
3. Immediately heat the sample at 85°C for 3 min.
4. Centrifuge at 12-17,000 x g for 2 min.
5. Load 10 μ l/lane on an appropriate SDS-polyacrylamide gel.
6. Load 10 μ l/lane Express-timator Standards in adjacent lanes.
7. Run the gel according to the manufacturer's instructions, stain with Coomassie blue, destain, and compare the expressed band to the Express-timator Standards to estimate the target protein expression level.

EXAMPLE OF SDS-PAGE WITH EXPRESS-TIMATOR STANDARDS

Samples (10 μ l) of the Express-timator Standards were run on a Bio-Rad Criterion™ 10-20% gradient gel and stained with Coomassie blue. Size Markers (M; Perfect Protein™ Markers 10-225 kDa) were run in an adjacent lane. Numbers below the Express-timator Standards correspond to mg/ml target protein.



ESTIMATION OF TARGET PROTEIN CONCENTRATION

Cell lysates prepared with BL21-derived *E. coli* host strains can be expected to contain 10.5 - 12.5 mg/ml total protein, 8 - 10 mg/ml soluble protein, and up to 5.5 mg/ml recombinant target protein, depending upon the expression efficiency.

The Express-timator Standards correspond to soluble cell lysate at a total protein concentration of 10 mg/ml, including the target protein. The target protein concentrations in the standards and their percentages of the total protein are given in the following table.

Standard	[52.2-kDa Target]	% Total Protein
0	0	0%
0.5	0.5 mg/ml	5%
1.0	1.0 mg/ml	10%
2.0	2.0 mg/ml	20%
3.0	3.0 mg/ml	30%

If the above protocol is followed the 10- μ l sample load and Express-timator Standards contain ~25 μ g total protein.

STORAGE

Store kit components at -20°C.

* Soluble cell lysate preparation (per g wet cell paste): re-suspend cell pellet in 5 ml SembaSonic™ Master Mix (or 5 ml SembaSonic Protein Extraction Reagent plus 10 μ l Soni-case™), incubate 15 min at room temperature, add 5 ml deionized water, mix, and centrifuge at 9,000 x g for 10 min. Supernatant is soluble cell lysate.

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