

# Express-timator™ Kit



Cat. No. R1001K

For more detailed information please refer to the Semba Biosciences website [www.sembabio.com](http://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

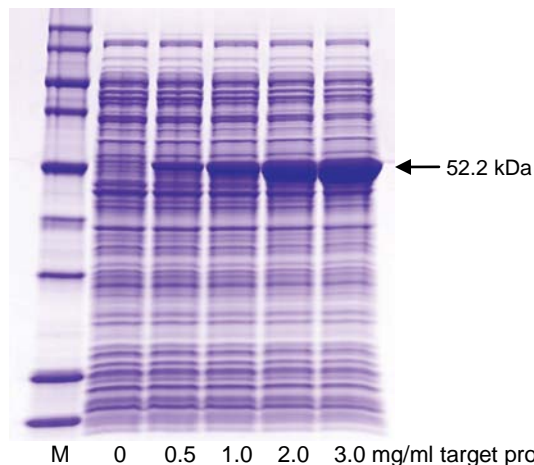
- # R1020E Express-timator Standard 0 mg/ml
- # R1021E Express-timator Standard 0.5 mg/ml
- # R1022E Express-timator Standard 1.0 mg/ml
- # R1023E Express-timator Standard 2.0 mg/ml
- # R1024E Express-timator Standard 3.0 mg/ml
- # R1025E SDS Sample Buffer, 4X

## 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  $\mu$ l lysate with 50  $\mu$ l deionized water and 25  $\mu$ 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  $\mu$ l/lane on an appropriate SDS-polyacrylamide gel.
6. Load 10  $\mu$ 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  $\mu$ 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 TRATION

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- $\mu$ l sample load and Express-timator Standards contain ~25  $\mu$ 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  $\mu$ 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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[www.sembabio.com](http://www.sembabio.com)

Toll-free 866.634.1114

TB101.01

Fax 608.441.8329