Protein Purification Using FastPROTEIN™ BLUE Protocol

Revision No.: 6550-X00B-9F01W

Catalog # 6550-400,6550-500

1. Grow cell culture to desired OD and induce for pre-determined time. Spin down culture and resuspend in the appropriate amount of ice cold 1X PBS or desired user provided lysis buffer. See table below for appropriate buffer volumes.

2. Add the appropriate volume of resuspended cells (see table below) to a FastPROTEIN BLUE tube and homogenize using a FastPrep® Instrument. The FastPROTEIN BLUE tubes should be pre-chilled on ice prior to homogenization. A speed of 6.0 for 20 seconds works very well for most *E. coli* strains. While using the FastPrep instrument gives the most complete lysis, continuously vortexing the tube for 5 minutes at 4°C can often release enough protein for subsequent tests. Vortexing can also be done at room temperature but results are dependent on the stability of your protein.

3. After homogenization, spin the tube in a microcentrifuge at 10 g for 1 minute at 4°C and transfer the supernatant to a new tube.

4. Analyze 20 µl of the supernatant on a polyacrylamide gel.

5. Supernatant from step three can be used for further purification.

Culture Volume	Amount of Lysis Buffer	Volume of lysate per FastPROTEIN® Blue tube
2-5 ml	300 ml	300 ml
10 ml	600 ml	600 ml
25 ml	2 ml	1 ml
50 ml	5 ml	1 ml

Note: Do not add more than 1.2mls total volume to the FastPROTEIN® BLUE tube.