Protein Purification Using FastPROTEIN™ RED Protocol

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Catalog # 6550-600,6550-700

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 Yeast Breakage Buffer (YBB). (Add protease inhibitors if desired). See table below for YBB volumes.

2. Add the appropriate volume of resuspended cells (see table below) to a pre-chilled FastPROTEIN RED tube and homogenize at a speed of 6.0 for 20 seconds. 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.

Culture Volume	Amount of YBB	Volume of lysate per Fast PROTEIN RED tube
2-5 ml	300 µl	300 µl
10 ml	600 µl	600 µl
25 ml	2 ml	1 ml
50 ml	5 ml	1 ml

5. Note: Do not add more than 1.2ml of total volume to the FastPROTEIN RED tube.

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