Cytosol MBP Sample Preparation

1. Add 50 ul of the overnight bacterial culture to 50 ml of LB medium-containing ampicillin (100 g/ml). Cultures were grown at 37 °C until the *A*600 reached 0.6 and then induced with 0.5 mM IPTG at 30 °C for 3 h.

2. The cells were harvested by centrifugation at 6000 rpm for 10 min. The pelleted cells were suspended in 5 ml buffer A (40 mM Tris-HCl pH 8.0, 100 mM NaCl). Then the cell were lysed by ultrasonic, and the cell lysate was separated by centrifugation at 13000 rpm for 15 min.

3. Add 50 ul SDS-PAGE loading buffer into 50 ul supernatant, then heat it at 95℃ for 10 min.

4. The pellet were suspended in 5 ml buffer A. Add 50 ul SDS-PAGE loading buffer into 50 ul suspension, then heat it at 95℃ for 30 min.

Periplasmic MBP Sample Preparation

1. Add 50 ul of the overnight bacterial culture to 50 ml of LB medium-containing Kanamycin (40 g/ml). Cultures were grown at 37 °C until the *A*600 reached 0.6 and then induced with 0.5 mM IPTG at 20 °C for 6 h.

2. The cell of 1 ml culture were harvested by centrifugation at 6000 rpm for 10 min. The pelleted cells were suspended in 100 ul buffer B (1 mg/ml lysozyme, 20%(m/v) sucrose, 30 mM Tris-HCl pH8.0, 1 mM EDTA), ice-bath for 20 min. The mixture were separated by centrifugation at 13000 rpm for 1 min.

3. Add 50 ul SDS-PAGE loading buffer into 50 ul supernatant, then heat it at 95℃ for 10 min.

4. The pellet were suspended in 5 ml buffer A. Add 50 ul SDS-PAGE loading buffer into 50 ul suspension, then heat it at 95℃ for 30 min.