NEBExpress Cell-free *E.coli* Protein Synthesis SDS-PAGE Protocol

Overview

In vitro protein synthesis reactions produced by the NEBExpress™ Cell-free *E coli* Protein Synthesis System can be directly loaded onto an SDS-PAGE gel without the need for acetone or TCA precipitation.

1. Combine 2 µL of a NEBExpress™ Cell-free *E. coli* Protein Synthesis System reaction with 6 µL of SDS-PAGE Blue Loading Buffer (NEB #B7703), and 10 µL H_2O. Also prepare a negative control sample.

2. Incubate at 100°C for 3-5 minutes.

3. Load 3 µL of the Unstained Protein Standard (NEB #P7717) into the first lane.

4. After a quick microcentrifuge spin, load samples directly on to the gel. To ensure uniform mobility, load an equal volume of SDS-PAGE Blue Loading Buffer into any unused wells.

5. Run the gel according to the manufacturer's recommendations.

6. Stain with Coomassie Blue or another stain as directed or proceed to Western Blot.

After staining, the target protein is typically observed as a unique band, absent in the negative control reaction. However; sometimes, the target has the same apparent molecular weight as an endogenous protein. In this case, the target protein will enhance or "darken" the co-migrating band.