PURExpress In Vitro Protein Synthesis Reaction Protocol

Components:

- Solution A: Energy solution
- Solution B: 36 Proteins + Ribosome

1. Thaw the necessary number of aliquots of solution A and B on ice. Pulse-spin in microfuge to collect solutions to bottom of tube.

2. Assemble the reaction on ice in a new tube in the following order:

Reagent	Volume
Solution A	4 µl
Solution B	3 µl
Template DNA	50 ng
MQ water	Up to 10 µl
Total	10 µl

3. Mix gently and pulse-spin in microfuge to collect mixture at the bottom of the tube.

4. Incubate at 37°C for 2-4 hours.

5. Analyze by method of choice or freeze at -20°C for later use.

6. For SDS-PAGE:

    - Mix 2.5 µL of sample, 2.5 µL MQ water, and 5 µL 2× SDS-PAGE sample buffer.
    - Incubate at 95 °C for 5 min.
    - Briefly spin down and load 8 µL onto the gel.

NEBExpress Cell-free *E. coli* Protein Synthesis Reaction Protocol

Components:

- NEBExpress™ S30 Synthesis Extract
- Protein Synthesis Buffer (2X)
- RNase Inhibitor, Murine
- T7 RNA Polymerase

1. Thaw all components on ice.

2. Gently vortex the NEBExpress® S30 Synthesis Extract and Protein Synthesis Buffer to mix.

3. Combine reagents in a PCR tube on ice as follows:

Reagent	Volume
NEBExpress® S30 Synthesis Extract	2.4 µl
Protein Synthesis Buffer (2X)	5 µl
T7 RNA Polymerase	0.2 µl
RNase Inhibitor, Murine	0.2 µl
Plasmid template (>100 ng/µl)	50 ng
MQ water	Up to 10 µL
Total	10 µl

4. Incubate reactions at 37°C, with vigorous shaking, for 2-4 hours.
5. Analyze by method of choice or freeze at -20°C for later use.
6. For SDS-PAGE:
    - Mix 2.5 µL of sample, 2.5 µL MQ water, and 5 µL 2× SDS-PAGE sample buffer.
    - Incubate at 95 °C for 5 min.
    - Briefly spin down and load 8 µL onto the gel.

Reference: NEB.com