

## OnePot PURE Expression Protocol

1. To prepare the starter cultures, combine 20 mL of LB media with 20  $\mu$ L of AMP (100 mg/ml). In a sterile 96, 1.3 mL deep-well plate, add 300  $\mu$ L of the media into 35 wells. Inoculate each of them with its respective strain, except elongation factor thermo unstable (EF-Tu), and seal the plate with a breathable membrane.
2. For the EF-Tu culture, inoculate 3 mL of LB media in a 14 mL culture tube with a snap cap. A single 3 mL of culture for EF-Tu is sufficient for one OnePot expression culture.
3. Incubate at 37 °C with shaking at 260 rpm overnight.
4. Transfer 200 mL of LB media and 200  $\mu$ L of AMP (100 mg/ml) into the sterile baffled flask.
5. Inoculate the OnePot PURE culture with 670  $\mu$ L of the EF-Tu culture and 22  $\mu$ L of each of the cultures from the deep-well plate.
6. Incubate the culture for 2 h at 37 °C with a shaking of 260 rpm.
7. Induce the culture with 200  $\mu$ L of 0.1 mM IPTG and grow for an additional 3 h.
8. Harvest the cells by centrifugation at 4 °C and 3220 x g for 10 min, and store the cell pellet at -80 °C until further use.