Quick Start: PandaPure™ Protein Systems (E. coli)

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Applicable products: PK002, PK006

PandaPure™ Protein Systems (E. coli) support recombinant protein expression, tag removal, and protein recovery using synthetic organelle targeting in vivo.

1. Before You Begin

Review SDS and standard lab safety requirements before use.

Wear PPE (lab coat, gloves, eye protection).

Prepare PandaPure Protein Reagent with PandaPure Protein Tag Cleaver + PandaPure Protein Buffer (HEPES), or another compatible buffer at pH 8–9.

Do not use prepared reagents if precipitation is visible.

2. Materials — What You Need

Plasmids:

- Organelle plasmid pTEAR-2;
- Expression vector pPEV-# (for example pPEV-1 available at PK006).

Consumables:

- Competent BL21(DE3), cloning reagents, sterile consumables.
- Culture components: Growth medium, kanamycin, chloramphenicol, IPTG, and optional anhydrotetracycline (aTc).
- Lysis reagent: Bacterial lysis reagent (Cat. LR050, LR250, LR500, or other common reagents).
- Tag-removal/recovery reagent: PandaPure Protein Reagent (Cat. RM001, RM002, RM003).

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3. First-Run Workflow (1 mL)

Cloning

- Insert your gene of interest into pPEV-# to generate the expression plasmid pPEX-POI.

Transformation

- Co-transform pTEAR-2 and pPEX-POI (250 ng each) into 50 µL BL21(DE3);
- Plate, and grow overnight at 37°C.

Expression

Stringent workflow

- Inoculate 1 mL culture with antibiotics;
- Incubate at 37°C until OD600 reaches 0.4–0.6;
- Induce with 100 µM IPTG and 100 ng/mL aTc;
- Incubate 3–48 h at 25–37°C, 220 rpm tube / 900 rpm deep-well.

"Easy" workflow

- Inoculate 1 mL culture with antibiotics;
- Incubate 24–48 h at 25–37°C, 220 rpm tube / 900 rpm deep-well.

Cell lysis

- Pellet (10,000 rpm, 10 min), discard supernatant;
- Resuspend in 200 µL lysis reagent, and incubate 20–30 min on a rotator or shaker;
- Pellet again (10,000 rpm, 10 min), and remove residual supernatant.

Tag removal and protein recovery

- Resuspend pellet in 200 µL PandaPure Protein Reagent (1x);
- Incubate 24 h at 25–37°C, centrifuge (10 min), collect supernatant.

4. Quick Checks

Observation	Action
No colonies	Check competent-cell quality and DNA quality.
	Consider sequential transformation (pTEAR-2 first, then pPEX-POI).
Low expression	Tune temperature, induction condition, and incubation time.
No cleavage	Prepare fresh reagent; verify pH 8–9, 25–37°C, and 16–24 h cleavage window; extend incubation if needed.
Low release	Re-elute pellets with compatible buffers and optimize pH/buffer/salt conditions.
	High-pressure filtration can be evaluated when appropriate.

For full troubleshooting, see User Guide Ver1.03.

5. Related Products

PandaPure Protein Systems (Cat. PK002, PK006)

PandaPure Protein Reagent (Cat. RM001, RM002, RM003)

PandaPure Protein Vector Sets (Cat. VP001, VP003, VP004)

6. Storage

Store DNA at -20°C to -80°C for long-term storage.

Store PandaPure Protein Reagent components at ambient temperature for up to 12 months.

Store prepared PandaPure Protein Reagent at ambient temperature to 4°C for weeks, or -20°C for months. Do not use reagent if precipitation is visible.

7. References

Guo, H., & Chen, J. (2024). Synthetic organelles enable protein purification in a single operation. bioRxiv, 2024-05. https://doi.org/10.1101/2024.05.17.594729

Guo, H., Ryan, J. C., Song, X., Mallet, A., Zhang, M., Pabst, V., et al. (2022). Spatial engineering of E. coli with addressable phase-separated RNAs. Cell, 185(20), 3823–3837. https://doi.org/10.1016/j.cell.2022.09.016

8. Support

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