**Description:** The PURExpress® Disulfide Bond Enhancer (PDBE) is a proprietary blend of proteins and buffer components designed to correctly fold target proteins with multiple disulfide bonds produced in PURExpress reactions. Added at the beginning of a reaction, the components promote a proper disulfide bond pattern by assisting with the oxidation of cysteine thiols and correcting mis-oxidized substrates. These enhancements can increase the yield of soluble and functionally active protein.

**Source:** Each of the recombinant proteins present in the PURExpress Disulfide Bond Enhancer has been expressed in *E. coli*.

Supplied in: 50 mM HEPES, pH 7.6, 100 mM KCl, 1 mM EDTA and 10% glycerol.

**Application:**
- Formation of properly folded proteins containing multiple disulfide bonds when produced by PURExpress.

**Reagents Supplied:**
- PURExpress Disulfide Bond Enhancer 1
- PURExpress Disulfide Bond Enhancer 2
- PURExpress Disulfide Bond Enhancer 3
- PURExpress Disulfide Bond Enhancer 4

**Reaction Conditions:**
Add 1 µl of each component to a PURExpress reaction (as part of the user-added 7.5 µl volume for templates and additives) and incubate reaction at 37°C for at least 2 hours.

**Notes on Use:** PURExpress Disulfide Bond Enhancer has been optimized for use with NEB PURExpress Systems (NEB #E6800, E3313). However, it is compatible with bacterial S30 lysate-based IVTT systems from other suppliers (Fig. 2). As the formulation of other systems varies, all guidelines, protocols and FAQs related to PDBE are for use with the PURExpress System.

**Protocol:**
1. Please refer to the PURExpress *In Vitro Protein Synthesis Kit manual* (NEB #E6800) for details about PURExpress reactions.
2. Plan number of and size of PURExpress reactions to be performed and number to be supplemented with PURExpress Disulfide Bond Enhancer.
3. During set-up of PURExpress reactions, add 1 µl of PURExpress Disulfide Bond Enhancer 1 and 1 µl of PURExpress Disulfide Bond Enhancer 2 per 25 µl of PURExpress reaction. If reactions larger or smaller than 25 µl are to be performed, the amount of the PURExpress Disulfide Bond Enhancer added should be scaled accordingly.
4. Allow reaction to proceed for at least 2 hrs at desired temperature (usually 37°C).
5. Place reactions on ice if they will be analyzed within 3-4 hrs; otherwise store at –20°C until needed.

**Companion Products:**
- PURExpress® *In Vitro Protein Synthesis Kit* #E6800S 10 reactions
- PURExpress® *In Vitro Protein Synthesis Kit* #E6800L 100 reactions
- PURExpress® *Δ Ribosome Kit* #E3313S 10 reactions
- PURExpress® *Δ RF123 Kit* #E850S 10 reactions
- *E. coli Ribosome* #P0763S 1 mg