PURExpress® Disulfide Bond Enhancer

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E6820S 0041305150511

E6820S

50 reactions **Lot: 0041305** **Exp: 5/15**

RECOMBINANT **Store at –20°C**

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

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.

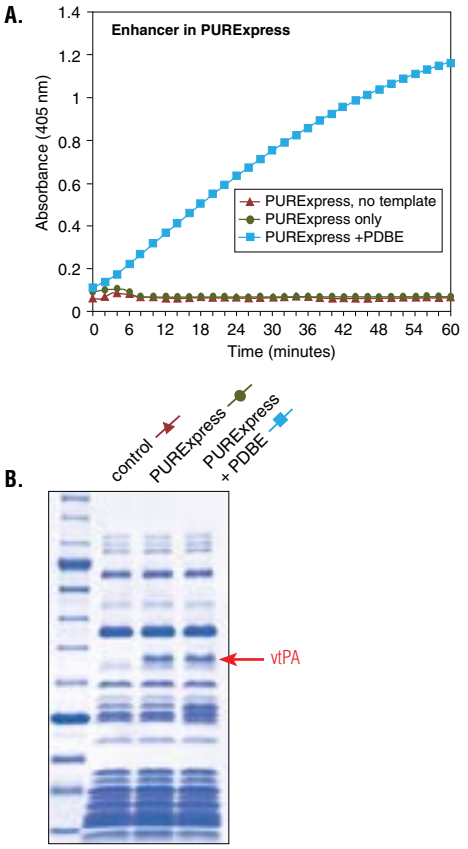

Figure 1: *PURExpress Disulfide Bond Enhancer (PDBE) promotes proper folding of active vtPA, a truncated version of tissue plasminogen activator with 9 disulfide bonds, 8 of which are non-consecutive.*

(A.) Reactions were set-up according to PURExpress specifications with the vtPA template DNA. After a two-hour incubation at 37°C, 5 µl of each reaction was used in an activity assay and cleavage of the chromogenic substrate was monitored for one hour. (B.) 2.5 µl of each reaction was resolved by SDS-PAGE and the gel stained with Coomassie Blue. The vtPA target protein is marked by a red arrow.

The addition of PDBE generates active protein. The difference in observed activity is due to disulfide bond folding, as measured by functional activity of the target, and is not due to differences in the amount of protein produced by PURExpress.

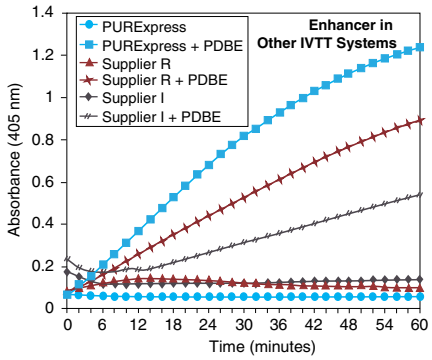

Figure 2: *PURExpress Disulfide Bond Enhancer is optimized for use in PURExpress but also shows utility in S30 lysate-based IVTT kits from other suppliers. Reactions were set-up according to manufacturer's specifications with equivalent amounts of template DNA encoding a truncated version of tissue plasminogen activator (9 disulfide bonds, 8 non-consecutive). After a two-hour incubation, 5 µl of each reaction was used in an activity assay and cleavage of the chromogenic substrate was monitored for one hour.*

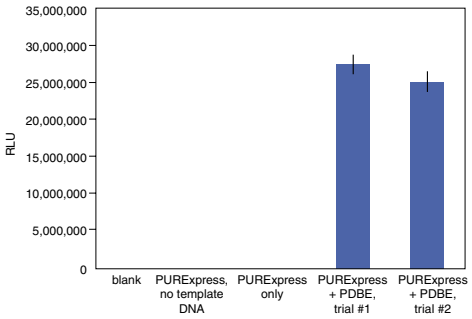

Figure 3: *PURExpress Disulfide Bond Enhancer promotes proper folding of active Gaussia Luciferase (GLuc) which contains 5 probable disulfide bonds. Reactions were set-up according to PURExpress specifications with equivalent amounts of template DNA encoding GLuc. After a two-hour incubation at 37°C, triplicate aliquots (2.5 µl) of each reaction were incubated with 50 µl of the GLuc substrate solution and the relative luminescence recorded after a 4 second counting window.*

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
#E6800L 100 reactions

PURExpress® Δ Ribosome Kit
#E3313S 10 reactions

PURExpress® Δ RF123 Kit
#E6850S 10 reactions

E. coli Ribosome
#P0763S 1 mg

CERTIFICATE OF ANALYSIS