EasyXpress® Disulfide *E.coli* Kit (10)

The EasyXpress Disulfide *E.coli* Kit (10) (cat. no. 32572), including buffers and reagents, should be stored immediately upon receipt at –80°C in a constant-temperature freezer.

For more information, please refer to the *EasyXpress Disulfide* E. coli *Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- This protocol is for expression of recombinant, disulfide-bonded proteins and protein complexes, especially Fab and scFv antibody fragments, using linear templates or plasmid DNA and the EasyXpress Disulfide *E. coli* Kit.
- For template design, refer to "Expression templates" in the *EasyXpress Disulfide* E. coli *Handbook*.
- A thermomixer (Eppendorf) or an incubator is required.
- All centrifugation steps should be carried out briefly at low centrifugal force (<1000 x g) unless stated otherwise.
- The in vitro translation system is extremely sensitive to nuclease contamination. Wear gloves and use RNase- and DNase-free reaction tubes and pipet tips with filters.
- EasyXpress Disulfide *E. coli* Extract is provided as two individual aliquots in single tubes (2 x 220 µl). Once thawed, use *E. coli* extracts within 4 h.
- Except for the actual transcription/translation incubation, all handling steps should be performed on ice. For protein synthesis reactions, add the components in the order given in the protocol and Table 1.
- After incubation for 4 h, reactions are boosted with EasyXpress Disulfide *E. coli* Folding Buffer to enhance disulfide bond formation. Usually, a total incubation time of 6 h is sufficient for disulfide bond formation. In some cases, extending the incubation after the boost for up to 20 h may help to ensure completeness of disulfide bond formation.
- If using PCR products generated with EasyXpress Linear Template Kits as templates, thaw XE-Solution provided by these kits on ice.

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1. Thaw and store EasyXpress Disulfide *E. coli* Extract and EasyXpress Disulfide *E. coli* Reaction Buffer on ice. Thaw RNase-free water at room temperature (15–25°C). Gently mix the EasyXpress Disulfide *E. coli* Extract and centrifuge before use. Make sure the EasyXpress Disulfide *E. coli* Reaction Buffer is completely thawed and fully dissolved. Table 1 provides a pipetting scheme for reaction setup.

2. Add 45 µl EasyXpress Disulfide *E. coli* Reaction Buffer to each tube of EasyXpress Disulfide *E. coli* Extract.

3. Mix and centrifuge briefly to collect reactions at the bottom of the tubes.

4. **Plasmid DNA template (A):** For each reaction, prepare a plasmid DNA expression template solution containing 2 µg of plasmid in a volume of 10 µl of RNase-free water. If expressing Fab heavy and light chains from separate plasmids, mix 1 µg each of the heavy chain plasmid and the light chain plasmid in a total volume of 10 µl of RNase-free water. This corresponds to a plasmid solution with a concentration of 0.1 µg/µl of each plasmid.

   PCR product template (B): For each reaction add 4 µl XE-Solution to ~2 µg DNA from the second PCR in a volume of 10 µl (performed using EasyXpress Linear Template Kits) in a separate tube and mix by pipetting. If expressing Fab heavy and light chains from separate PCR products, add a total of 2.5 µg PCR product.

5. Add 10 µl plasmid DNA expression template mix (step 4; Plasmid DNA template) or 10 µl XE-Solution–PCR product mix (step 4; PCR product template) to EasyXpress *E. coli* Disulfide Extract. Mix and briefly centrifuge to collect reactions.

6. Calculate the amount of RNase-free water needed to obtain a final reaction volume of 140 µl and add to the mix from step 5. Mix and centrifuge briefly to collect reactions. Incubate the reactions for 4 h at 27°C with optional shaking at 500 rpm in a thermomixer. Thaw EasyXpress Disulfide *E. coli* Folding Buffer at room temperature.

7. Boost each expression reaction after 4 h by adding 30 µl of EasyXpress Disulfide *E. coli* Folding Buffer to a final volume of 170 µl, mix and centrifuge briefly. Incubate for an additional 2–20 h at 27°C.

8. Proceed to "Sample analysis" or store samples at –80°C until ready for analysis.

Table 1. Pipetting scheme for setup of EasyXpress Disulfide *E. coli* protein synthesis reactions

Reagent/component	In vitro translation samples	Positive control	No template control
EasyXpress Disulfide *E. coli* Extract	44 µl	44 µl	44 µl
EasyXpress Disulfide *E. coli* Reaction Buffer	45 µl	45 µl	45 µl
Add plasmid DNA template or PCR product template			
A Plasmid DNA template	2 µg/10 µl	10 µl	-
B PCR product/linear template	Approx. 2 µg*	Approx. 2 µg*	-
B XE-Solution†	4 µl	4 µl	-
RNase-free water	Add to 140 µl	41 µl	51 µl
Total (before boost)	140 µl	140 µl	140 µl
Incubate for 4 h at 27°C (with optional shaking at 500 rpm)			
EasyXpress Disulfide *E. coli* Folding Buffer	30 µl	30 µl	30 µl
Total	170 µl	170 µl	170 µl
Incubate for additional 2–20 h at 27°C (with optional shaking at 500 rpm)			

* If expressing Fab heavy and light chain from separate PCR products, add a total amount of ~1.25 µg PCR product per chain (approx. 8.5 µl of a PCR product concentrated at 150 ng/µl).

† XE-Solution should be used only when PCR templates from EasyXpress Linear Template Kits are used. Mix XE-Solution with the second PCR product before adding to the reaction. It is important that XE-Solution is not added directly to the diluted *E. coli* extract.

EasyXpress Disulfide *E. coli* Positive Control DNA (yellow screw-cap) is supplied with the kit. The heavy chain of the synthesized anti-CD4 Fab that serves as a positive control contains a C-terminal 6xHis tag.

Sample analysis

1. Centrifuge expression reactions for 15 min at 16,000 x g at room temperature (15–25°C). If expression reactions have been stored at –80°C, thaw samples before centrifugation.

2. Transfer the supernatant (soluble protein fraction) into a fresh microcentrifuge tube. Store on ice, if required. If the insoluble protein fraction is to be analyzed, resuspend the pellet in 170 µl PBS per reaction containing 0.5% Triton® X-100 by vigorously vortexing or pipetting. For western blot analysis, use 5–10 µl of the supernatant or the resuspended pellet per gel lane. For ELISA assays, perform serial dilutions of the soluble fraction starting at 1:100.