EasyXpress® Disulfide *E. coli* Kit (96)

The EasyXpress Disulfide *E. coli* Kit (96) (cat. no. 32578), 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 the expression of multiple 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 PCR cycler, stepper pipet, and centrifuge with a rotor for 96-well plates are required.
- 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 in four individual tubes, each containing 1160 µl. Once thawed, use *E. coli* extracts within 4 h. If required, the EasyXpress Disulfide *E. coli* Kit (96) can be used for just 24 reactions. Use one tube of EasyXpress Disulfide *E. coli* Extract to prepare a volume of master mix for 24 reactions, including an excess of 10%.
- One 96-well plate and silicone mat are provided with the EasyXpress Disulfide *E. coli* Kit (96). For 24 reactions, place tape over the empty wells.
- Additional plates can be ordered from nerbe plus GmbH (cat. no. 040830140).
- Except for the 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.

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- 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 time for up to 20 h after the boost may help ensure completeness of disulfide bond formation.
- If using PCR products generated with EasyXpress Linear Template Kits as templates, thaw XE-Solution on ice. XE-Solution is provided with EasyXpress Linear Template Kits.

1. Dilute linear expression templates or plasmids for the number of reactions required in the 96-well plate provided. If necessary, the plate can be tightly sealed and stored at 4°C overnight.

   Plasmid DNA template: For each reaction, prepare a plasmid DNA expression template solution containing 2 µg of plasmid in 24 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 24 µl of RNase-free water.

   PCR product template: For each reaction add 4 µl XE-Solution to ~2 µg DNA from the second PCR (performed using EasyXpress Linear Template Kits) in a final volume of 24 µl, and mix by pipetting up and down. If expressing Fab heavy and light chains from separate PCR products, add a total of 2.5 µg PCR product.

2. The EasyXpress Disulfide *E. coli* Kit (96) can be used to prepare a master mix for 24 samples. Use one tube of EasyXpress Disulfide *E. coli* Extract to prepare a volume of master mix for 24 reactions, including a 10% excess. Add the required volumes of EasyXpress Disulfide *E. coli* Reaction Buffer and RNase-free water as specified in Table 1.

3. Thaw and store EasyXpress Disulfide *E. coli* Extract and EasyXpress Disulfide *E. coli* Reaction Buffer on ice. Make sure not to invert the tube containing the EasyXpress Disulfide *E. coli* Extract. Thaw RNase-free water at room temperature (15–25°C). Gently mix the EasyXpress Disulfide *E. coli* Extract by pipetting before use. Make sure that the EasyXpress Disulfide *E. coli* Reaction Buffer is completely thawed and fully dissolved.

4. Calculate the amount of template, XE-Solution, and water needed to obtain a final volume of 24 µl for the template mix. Table 1 provides a pipetting scheme for reaction setup.

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

Reagent/component (for 24 reactions, including 10% excess)	Plasmid DNA template	PCR product/linear template	Positive control	No template control
Add plasmid, linear template, positive control, or RNase-free water for no template control to 96-well plate				
Template DNA	2 µg/24 µl per well	Approx. 2 µg	10 µl in one well	–
XE-Solution*	–	4 µl per well†	–	–
Add RNase-free water to bring the volume to 24 µl per well				
Preparation of master mix for 24 reactions				
EasyXpress Disulfide *E. coli* Extract		1160 µl		
EasyXpress Disulfide *E. coli* Reaction Buffer		1188 µl		
RNase-free water		712 µl		
Total master mix volume		3060 µl		
Volume of master mix per well		116 µl		
Total reaction volume per well (before boost)		140 µl		
Incubate for 4 h at 27°C				
EasyXpress Disulfide *E. coli* Folding Buffer		30 µl per well		
Total		170 µl per well		
Incubate for an additional 2–20 h at 27°C				

* 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.

† 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).

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.

5. Add all components in the given order and mix the master mix by pipetting.

6. Using a stepper pipet, pipet the master mix into the 96-well plate containing the templates.

7. Incubate the reactions for 4 h at 27°C in a PCR cycler. Thaw EasyXpress Disulfide *E. coli* Folding Buffer at room temperature.

8. After incubation for 4 h, boost each expression reaction by adding 30 µl of EasyXpress Disulfide *E. coli* Folding Buffer Mix and centrifuge briefly. Incubate for an additional 2–20 h at 27°C.

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

Sample analysis

1. Centrifuge expression reactions for 1 h at 4000 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) to a fresh 96-well plate (not supplied). Store on ice, if necessary. If the insoluble protein fraction is to be analyzed, resuspend the pellet in 170 µl PBS containing 0.5% Triton® X-100 by vigorous pipetting. For western blot analysis, use 5–10 µl of supernatant or suspended pellet per gel lane. For ELISA assays, perform serial dilutions of the soluble fraction starting at 1:100.