EasyXpress® Disulfide Insect Kit (10)

The EasyXpress Disulfide Insect Kit (10) (cat. no. 32582), including buffers and reagents, should be stored immediately upon receipt at –80°C in a constant-temperature freezer.

For further information, please refer to the *EasyXpress Disulfide Insect Handbook* and the *EasyXpress Linear Template Kit Plus 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. EasyXpress Disulfide Insect Kits are developed to perform coupled transcription/translation reactions.
- For template design, refer to "DNA templates" in the *EasyXpress Disulfide Insect Handbook* and "Strategy for designing gene-specific primers" in the *EasyXpress Linear Template Kit Plus Handbook*.
- A thermomixer (Eppendorf) or an incubator is required.
- All centrifugation steps should be carried out briefly (<5 seconds) at low centrifugal force (<500 x *g*), unless otherwise stated.
- 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 Insect Extract is provided in five individual tubes, each containing 70 µl. Once thawed, use EasyXpress Disulfide Insect Extract within 4 h.
- Once thawed, store the Brij-35 solution at 4°C.
- 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 in Tables 1–2.

October 2011

1. Thaw the EasyXpress Disulfide Insect Extract, EasyXpress Disulfide Insect Reaction Buffer, and EasyXpress Disulfide Insect Energy Mix on ice. Thaw RNase-free water at room temperature (15–25°C). Gently mix and centrifuge all components before use. Make sure the EasyXpress Disulfide Insect Reaction Buffer is completely thawed and fully dissolved. If a precipitate is visible warm the buffer at 37°C.
2. Pipet the components of the transcription/translation reactions in the order shown in Table 1 for plasmid DNA templates or Table 2 for linear templates (PCR product). Directly proceed with the addition of templates.

   Plasmid DNA template: For each reaction, prepare a plasmid DNA expression template solution containing 1.5 µg of plasmid in 10 µl of RNase-free water. If expressing Fab heavy and light chains from separate plasmids, the final concentration in the transcription/translation reaction is 0.75 µg/100 µl reaction.

   PCR product (linear template): Use the product from the second PCR step, without further purification, as a linear template for protein synthesis. For most linear templates, the optimal amount is 500 ng/100 µl reaction. If expressing Fab heavy and light chains from two separate linear templates, use 250 ng/100 µl reaction for each chain. The amount of linear template can be optimized, but the total volume of linear templates should not exceed 20 µl/100 µl protein synthesis reaction.
3. Mix briefly by vortexing (<5 seconds) and centrifuge briefly (<500 x *g*) to collect the reactions at the bottom of the tubes.
4. Incubate the cell-free transcription/translation reactions in a thermomixer or incubator for 4 h at 25°C. Thaw Brij-35 Solution on ice.
5. Proceed to "Sample analysis" or store samples at –80°C until ready for analysis.

Sample analysis

1. After protein synthesis, add 6 µl of EasyXpress Disulfide Insect Brij-35 Solution to each 100 µl protein synthesis reaction to lyse microsomes harboring the synthesized disulfide-bonded protein. If expression reactions have been stored at –80°C prior to analysis, thaw samples on ice before lysis.
2. Incubate for 5 min at room temperature with strong shaking (700–900 rpm). Do not vortex as this can lead to air bubbles.
3. Centrifuge the lysis reactions for 10 min at 15,000 x *g* at room temperature (15–25°C).

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

Table 1. Pipetting scheme for setup of EasyXpress Disulfide Insect protein synthesis reactions using plasmid DNA as template

Component	Disulfide protein or Fab synthesis reaction	Positive control reaction	No template control reaction
RNase-free water	25 µl	15 µl	35 µl
EasyXpress Disulfide Insect Reaction Buffer	10 µl	10 µl	10 µl
EasyXpress Disulfide Insect Extract	35 µl	35 µl	35 µl
Plasmid template 1.5 µg in 10 µl*	10 µl	–	–
EasyXpress Disulfide Positive Control DNA†	–	20 µl	–
EasyXpress Disulfide Insect Energy Mix	20 µl	20 µl	20 µl
Total	100 µl	100 µl	100 µl

* If expressing Fab heavy and light chains from separate plasmids, the final concentration should be 0.75 µg/100 µl reaction. In some cases, the protein yield can be improved by increasing the final plasmid concentrations up to 1.5 µg/100 µl reaction for each Fab chain.

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

Table 2. Pipetting scheme for setup of EasyXpress Disulfide Insect protein synthesis reactions using a linear template

Component	Disulfide protein or Fab synthesis reaction	Positive control reaction	No template control reaction
RNase-free water	Variable	15 µl	35 µl
EasyXpress Disulfide Insect Reaction Buffer	10 µl	10 µl	10 µl
EasyXpress Disulfide Insect Extract	35 µl	35 µl	35 µl
Linear template DNA	500 ng* (max. 20 µl volume)	–	–
EasyXpress Disulfide Positive Control DNA†	–	20 µl	–
EasyXpress Disulfide Insect Energy Mix	20 µl	20 µl	20 µl
Total	100 µl	100 µl	100 µl

* For most linear templates, the optimal amount of template is 500 ng/100 µl reaction. In some cases, an increase of up to 1.5 µg/ 100µl reaction might lead to a higher protein yield. For expression of Fab light and heavy chain, 250 ng/100 µl reaction for each Fab chain is optimal. In some cases, an increase of up to 500 ng/100 µl leads to a higher protein yield.

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