EasyXpress Random Biotin Kit

For random cotranslational labeling of recombinant proteins during cell-free protein synthesis

The EasyXpress Random Biotin Kit is used in combination with *E. coli*-based EasyXpress Protein Synthesis Kits (cat. nos. 32502 and 32506) and the EasyXpress Insect Kit II (cat. no. 32562) to generate biotin-labeled recombinant proteins.

In vitro translation is a widely used tool for the production of recombinant proteins. For small-scale analyses, synthesized proteins are usually visualized by detection of radioactively labeled amino acids incorporated during translation. However, incorporating radioactively labeled amino acids — such as [35S] methionine or [14C] leucine — is time-consuming, generates hazardous waste, and requires extra safety precautions. There is therefore a need for alternative, non-radioactive methods for labeling and detection of in vitro translated proteins.

QIAGEN offers the EasyXpress Random Biotin Kit for random cotranslational non-radioactive labeling of proteins. Using in vitro translation in *E. coli*- or insect-cell extracts biotin moieties are incorporated into recombinant proteins with high efficiency. The biotin moiety greatly facilitates detection of any recombinant protein using a universally applicable method. The EasyXpress Random Biotin Kit is superior to comparable solutions with respect to ease-of-use and efficiency of biotin incorporation.

The kit comprises a synthetic tRNA aminoacylated with lysine labeled at the epsilon position with biotin and carrying a phenylalanine GGA anticodon. This tRNA directs the incorporation of a biotin residue at phenylalanine UUC codons (Figure 1). The EasyXpress Random Biotin Kit is used in combination with *E. coli*-based EasyXpress Protein Synthesis Kits and the EasyXpress Insect Kit II to generate biotin-labeled recombinant proteins (Figures 2 and 3). To label a recombinant protein, biotinyl-lysyl tRNA is simply added to a standard EasyXpress protein synthesis reaction. During protein synthesis a few phenylalanine codons will be translated into biotinylated lysine in a random but highly efficient manner. Since the tRNA functions in prokaryotic and eukaryotic systems, it can be used in other cell-free systems (e.g., those based on wheat germ or reticulocytes). Biotin incorporation represents a universal, easy-to-use non-radioactive labeling method that enables high-sensitivity detection of recombinant proteins, for example using streptavidin conjugates following western blotting.

Kit Contents

EasyXpress Random Biotin Kit, cat. no. 32612	For 60 reactions
EasyXpress Biotinyl-Lysyl tRNA (Phe)	4 x 15 µl
Product Sheet	1

Table 1. Biotinyl-Lysyl tRNA in the EasyXpress Random Biotin Kit

tRNA	tRNA anticodon	mRNA codon	Replaced amino acid	Incorporated amino acid
Bio-Lys tRNA (Phe)	GAA	UUC	Phenylalanine	Biotinyl-lysine

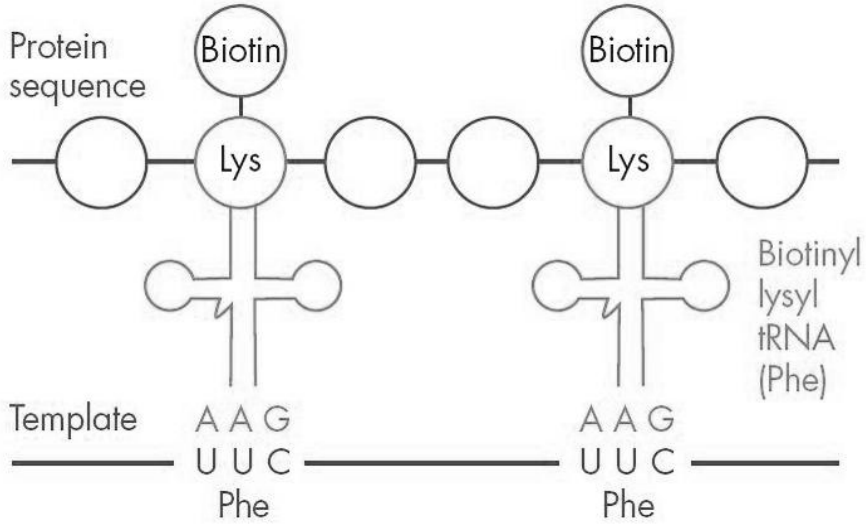

Figure 1. Insertion of biotinylated lysine residues at phenylalanine UUC codons.

Applications

At the beginning of projects where proteins are produced on an analytical scale (e.g., to check the efficiency of expression constructs or verify predicted protein size) it is often advantageous to label the synthesized protein in order to facilitate its detection after gel electrophoresis. Labeling proteins with biotin, a small molecule that binds avidin or streptavidin with high affinity, enables their detection in a rapid and straightforward non-radioactive procedure using avidin or streptavidin conjugates.

For directed immobilization or applications such as protein-protein interaction analysis using LiquiChip®, other xMAP®, or BIACORE® systems we recommend using the EasyXpress Site-Specific Biotin Kit (QIAGEN cat.no. 32602), which allows site-specific incorporation of biotin at a stoichiometry of 1:1 (i.e.., one biotin molecule per protein molecule).

Labeling protocols

If producing biotinylated proteins using the **EasyXpress Insect Kit II**, add 1 µl EasyXpress Biotinyl-Lysyl-tRNA (Phe) to each 50 µl translation reaction. It is important that the EasyXpress Biotinyl-Lysyl tRNA is added as the last reaction component, 10 minutes after starting the translation reaction. To enhance signal intensity in detection procedures, the proportion of incorporated biotin can be increased by adding up to 3 µl EasyXpress Biotinyl-Lysyl-tRNA (Phe) per reaction. A detailed protocol can be found in the *EasyXpress Insect Cell Protein Synthesis Handbook*, Third Edition.

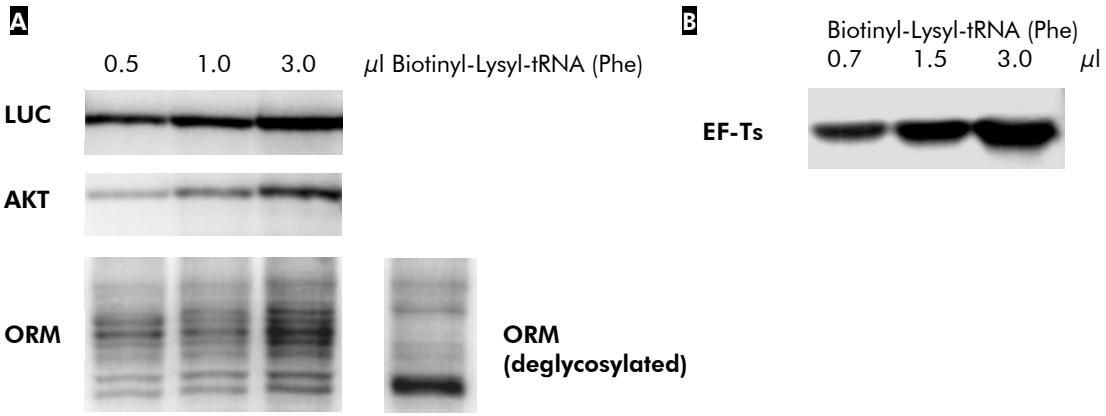

Figure 2. Efficient synthesis of biotinylated recombinant proteins. The indicated amount of Biotinyl-Lysyl-tRNA (Phe) was added to 50 µl translation reactions performed using **A** the EasyXpress Insect Kit II or **B** the EasyXpress Protein Synthesis Mini Kit. Biotinylated proteins were detected using streptavidin-peroxidase following western blotting. **LUC**: luciferase (control protein supplied with kit); **AKT**: human RAC-alpha serine/threonine kinase; **ORM**: human Alpha-1-acid glycoprotein 1, displaying multiple glycosylated forms. Treatment of ORM with PNGaseF produced deglycosylated protein, verifying compatibility of biotin labeling with glycosylation. **EF-Ts**: *E. coli* elongation factor Ts (control protein supplied with kit).

If producing biotinylated proteins using **EasyXpress *E. coli*-based kits**, add 1 µl EasyXpress Biotinyl-Lysyl-tRNA (Phe) to each 50 µl translation reaction. To enhance signal intensity in detection procedures, the proportion of incorporated biotin can be increased by adding up to 3 µl EasyXpress Biotinyl-Lysyl-tRNA (Phe) per reaction. It is important that the EasyXpress Biotinyl-Lysyl tRNA is the last reaction component added. A detailed protocol can be found in the *EasyXpress Protein Synthesis Handbook*.

Shipping and Storage

The EasyXpress Random Biotin Kit is shipped on dry ice and should be stored at –70°C. Once thawed, Biotinyl-Lysyl tRNA should be stored on ice and quickly returned to a –70°C freezer after use. Do not refreeze and thaw more than four times. When stored under the above conditions and handled correctly, the kit can be kept for at least 6 months without showing any reduction in performance.

Ordering Information

Product	Contents	Cat. no.
EasyXpress Random Biotin Kit	For 60 reactions: 4 x 15 µl EasyXpress Biotinyl-Lysyl tRNA (Phe)	32612
Related products		
EasyXpress Protein Synthesis Kit (20)*	For 20 x 50 µl reactions: E. coli extract, reaction buffer, RNase-free water, and positive-control DNA	32502
EasyXpress Insect Kit II (20)*	For 20 x 50 µl reactions: *Spodoptera frugiperda* insect cell extract, reaction buffers, in vitro transcription reaction components, RNase-free water, gel-filtration columns, and positive-control DNA	32562
EasyXpress Site-Specific Biotin Kit	For 5 x 25 µl reactions: E. coli extract, reaction buffer, RNase-free Water, biotinyl-lysyl tRNA (amber), and positive-control DNA	32602
EasyXpress Linear Template Kit Plus (20)	For 20 two-step PCRs: ProofStart DNA Polymerase, buffer, RNase-free water, Q-Solution, XE-Solution, positive-control DNA, and optimized PCR primers	32723

* Other kit sizes available, please inquire.

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