

Increasing the ease and speed of eukaryotic protein expression

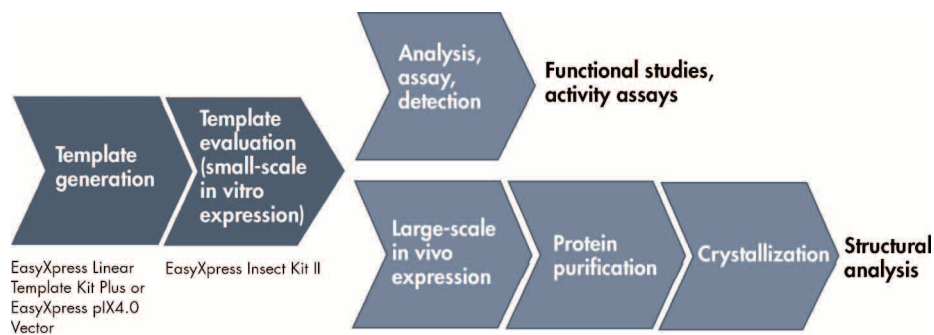


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Optimized expression of posttranslationally modified eukaryotic proteins

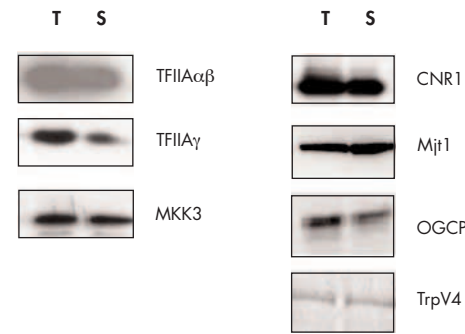
The EasyXpress Protein Synthesis Insect Kit was the first commercially available in vitro protein synthesis kit based on cell lysates derived from insect cell cultures. Thanks to an optimized lysate production procedure, this kit is now even more efficient. The homogeneous lysates enable robust and efficient expression of a wide range of proteins — e.g., clotting factors, protein kinases, membrane proteins, and transcription factors — with a range of post-translational modifications, such as glycosylation, phosphorylation, and signal-peptide cleavage (1, 2).



Soluble eukaryotic proteins from insect-cell extracts

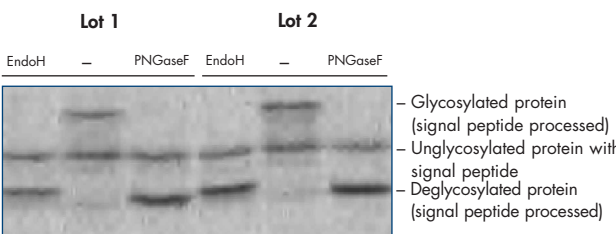
- Expression of soluble human proteins in *E. coli* can be problematic due to folding problems
- Expression of proteins in EasyXpress insect-derived extracts delivers highly soluble proteins, which were sparingly soluble when expressed in *E. coli*.

Soluble Eukaryotic Proteins



The indicated eukaryotic protein was expressed using the EasyXpress Insect Kit II. Equal volumes of total protein (T) corresponding to the crude reaction, and soluble protein (S) corresponding to supernatant after centrifugation (10 min, 15,000 x g), were separated by SDS-PAGE and detected using protein-specific antibodies after western blotting or by incorporation of ¹⁴C-labeled leucine and autoradiography (TrpV4).

Lot-to-Lot Reproducibility



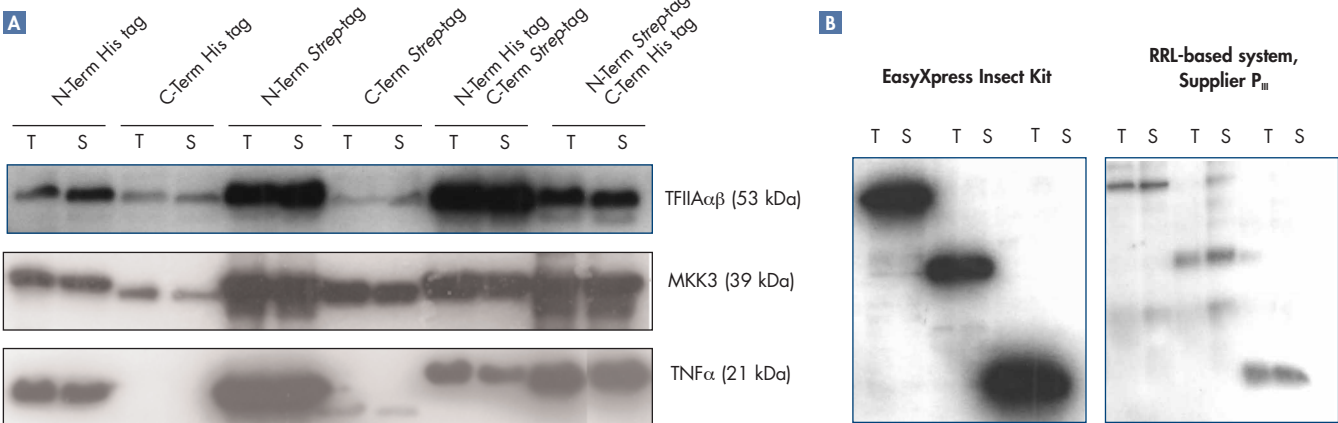
High lot-to-lot reproducibility ensures efficient glycosylation of eukaryotic proteins. ¹⁴C-labeled erythropoietin was synthesized using 2 different lots of EasyXpress Insect Kit cell extract. From the respective reactions, 8 µl aliquots were treated with the glycosidase EndoH or PNGaseF, or left untreated. Subsequently, proteins were separated by SDS-PAGE and visualized using a PhosphorImager. Glycosylated protein has its signal peptide removed prior to or during glycosylation: therefore deglycosylated protein has no signal peptide and greater electrophoretic mobility than unglycosylated protein, which still carries a signal peptide.

- Reproducibility of expression or level of post-translational modifications can vary greatly in other eukaryotic cell-based systems
- Insect cell lysates used in EasyXpress Insect Kits show high lot-to-lot reproducibility

Screening multiple constructs to optimize expression

- Expression yields and solubility can often be influenced by adding affinity tags to protein expression constructs
- Using the EasyXpress Linear Template Kit Plus enables generation of a range of PCR products with combinations of 6xHis- and *Strep*-tags at either terminus
- The new EasyXpress Insect Kit II is able to use PCR products as expression templates, meaning that multiple constructs can be quickly and easily screened to determine the optimal configuration before scale-up

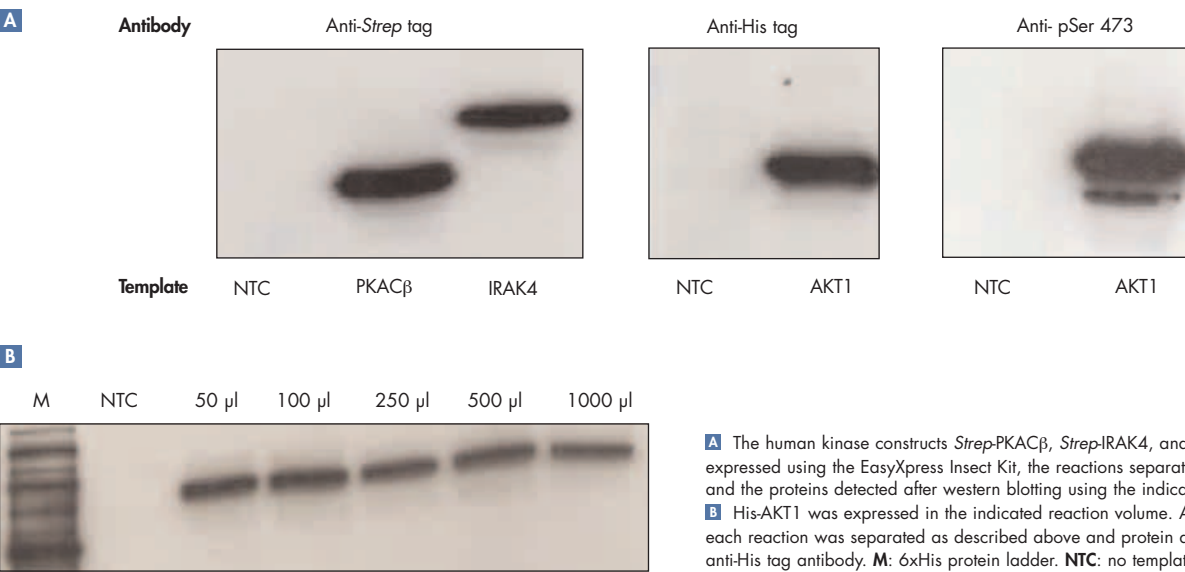
Fast and Easy Expression Construct Screening



A The new EasyXpress Insect Kit II enables fast and easy screening of expression constructs. TFIIAαβ, MKK3, and TNFα were expressed in parallel reactions with six different affinity tag combinations. 5 µl aliquots of total (T) or soluble (S) protein were separated on an SDS-PAGE gel, blotted onto a nitrocellulose membrane, and proteins detected by chemiluminescence using a mixture of anti-*Strep*-tag and anti-His-tag antibodies. **B** The performance of the optimal construct for each protein was compared in expression reactions using the EasyXpress Insect Kit and a rabbit reticulocyte lysate based system. 2.5 µl (EasyXpress) or 12.5 µl (RRL system) aliquots of the respective reaction were analyzed as before.

Expression of human kinases and linearity of yields

- Efficient expression of human kinases is often difficult to obtain using prokaryotic systems
- Kinases often require phosphorylation at a specific residue for activity
- Phosphorylation of serine residue 473 in AKT1, required for activity (3), could be demonstrated using an antibody specific for this modification
- AKT1 yields per ml reaction are constant over a range of reaction sizes up to 1 ml

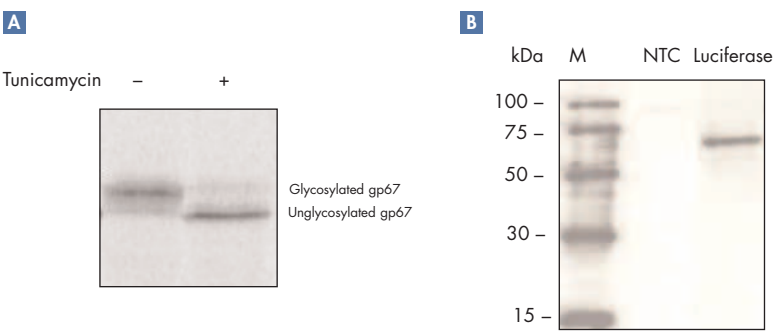


A The human kinase constructs *Strep*-PKACβ, *Strep*-IRAK4, and *His*-AKT1 were expressed using the EasyXpress Insect Kit, the reactions separated by SDS-PAGE, and the proteins detected after western blotting using the indicated antibody. **B** *His*-AKT1 was expressed in the indicated reaction volume. An equal volume of each reaction was separated as described above and protein detected using an anti-His tag antibody. **M**: 6xHis protein ladder. **NTC**: no template control.

Expression of functional eukaryotic proteins

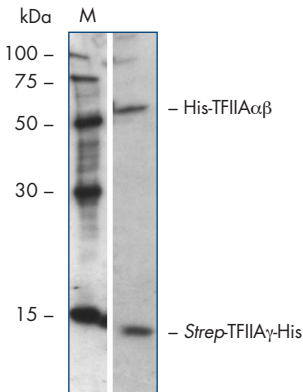
- Many eukaryotic proteins require posttranslational modifications to fold or function properly
- Prokaryotic expression systems lack the cellular machinery (e.g., ER) required to perform these modifications
- Insect-cell lysates in EasyXpress Insect kits contain the cellular elements required for a range of PTMs including glycosylation, phosphorylation, and signal-peptide processing (4)

Expression of Posttranslationally Modified Proteins



A The baculoviral glycoprotein gp67 was expressed using the EasyXpress Insect Kit in the presence (10 µg/ml reaction volume) and absence of the glycosylation inhibitor tunicamycin. **B** Firefly luciferase was expressed using the EasyXpress Insect Kit. Yields of luciferase were measured using a commercially available kit and found to be up to 40 µg active luciferase per ml reaction. **M**: markers; 6xHis Protein Ladder. **NTC**: no template control.

Pull-Down Assay Using In Vitro Expressed Transcription Factors



Pull-down assay shows interaction of two active, correctly folded transcription factors. The interaction partners TFIIAγ and TFIIAαβ were expressed using the EasyXpress Insect Kit II. The TFIIAγ protein carried an N-terminal *Strep*-tag and a C-terminal His tag. The TFIIAαβ protein carried an N-terminal His tag. *Strep*-Tactin Magnetic Beads were added to the TFIIAγ synthesis reaction lysate and the *Strep*-tagged TFIIAγ was allowed to bind to the beads. After a wash step, purified His-tagged TFIIAαβ was added. After another wash step, TFIIAγ-TFIIAαβ complexes were eluted using 10 µl *Strep*-Tactin elution buffer. After separation by SDS-PAGE and western blotting, proteins were detected using anti-His-tag antibodies and chemiluminescent detection. **M**: markers; 6xHis Protein Ladder.

Summary

- The EasyXpress Insect Kit enables the expression of a number of different proteins from numerous classes
- Expressed proteins are posttranslationally modified with high efficiency
- The expression procedure delivers reproducible yields and modifications

Protein family	Proteins successfully synthesized using the EasyXpress Insect System
Kinases	PKACβ, IRAK4, AKT1, MKK3, pKαα
Membrane proteins	Mitochondrial 2-oxoglutarate carrier protein (OGCP), Mj1, TrpV4, CNR1
Blood coagulation factors	Factor II, Factor VIII (B domain)
Transcription/translation factors	TFIIAαβ, TFIIAγ, eIF4E
Cytokines and growth factors	TNFα, TAU
Glycoproteins	EPO, alpha-1-acid glycoprotein 1 (ORM), gp67
Other enzymes	Luciferase, CAT, Isopentenyl-diphosphate delta-isomerase (IDI), β-glucuronidase, DHFR
Other proteins	GFP variants, FABP, Ubiquitin, Transthyretin

References

- [1] Kubick, S. et al. (2003) In vitro translation in an insect-based cell-free system. In Swartz, J.R. (Ed.) Cell-Free Protein Expression, 1st ed., Berlin, Springer Verlag, pp. 209-217.
- [2] von Groll, U. et al. (2006) Advances in insect-based cell-free protein expression. In Kudlicki, W. (Ed.) Cell-Free Protein Expression, 1st ed., Austin, RG Landes Co. in press.
- [3] Au et al. (2004) Insulin regulates hepatic apolipoprotein B production independent of the mass or activity of AKT/PKB alpha. Metabolism **53**, 228.
- [4] Kubick, S. et al. (2005) Insect-cell based in vitro synthesis of posttranslationally modified proteins. QIAGEN News 2005, **2**, 41.

Acknowledgements

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