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Poster Presentation

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Increasing the ease and speed of eukaryotic protein expression: a new cell-free in vitro translation system based on Sf insect cell extracts

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Background

For researchers looking for fast access to their protein of interest, cell-free expression systems are an attractive option, as they do not require specialized equipment, are open systems, and offer fast screening of expression constructs for expression efficiency, yield, solubility and other criteria (e.g. requirement of cofactors).

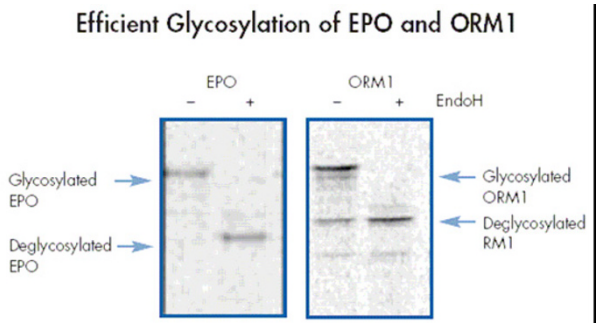

Figure 1
6xHis-tagged EPO (erythropoietin) and ORM1 (Alpha-1-acid glycoprotein 1) were expressed as ^{14}C-Leu-labeled proteins using the EasyXpress Protein Synthesis Insect Kit. After expression an aliquot of each was treated with the endoglycosidase EndoH, which removes glycan moieties form glycosylated proteins. Aliquots of treated and untreated protein separated by SDS_PAGE and proteins visualized by autoradiography.

A broad range of eukaryotic proteins require posttranslational modifications such as phosphorylation, glycosylation, or signal peptide cleavage to display full functional activity.

Results

We will describe the most important features of a new system for recombinant eukaryotic protein expression that retains the speed and economy inherent to cell-free methods. Data showing the ability of the system to generate complex posttranslational modifications (PTMs), including highly efficient glycosylation of EPO and ORM1 pro-

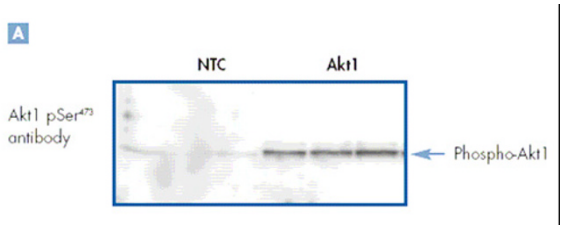

Figure 2
Akt1 was expressed in triplicate reactions using the EasyXpress Protein Synthesis Insect Kit. 2.5 µl aliquots were separated by SDS-PAGE and protein detected by Immunoblotting using an antibody specific for akt1 phosphorylated at Ser 473. NTC: no template control; M: marker.

teins (Figure 1) and phosphorylation of AKt1 kinase (Figure 2) will be presented. The robustness of the system is demonstrated by the expression of membrane proteins (e.g., OGCP) and the synthesis of several proteins, such as human clotting factors, that could not be expressed in *E. coli* systems.

Conclusion

Expressed proteins with the EasyXpress Protein Synthesis Insect Kit are post-translationally modified with high efficiency.

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