Monitoring the Expression, Processing, and Purification of GST-Tagged Proteins Using the Experion™ Automated Electrophoresis System

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Introduction

Expression and purification of recombinant proteins is often a prerequisite for their structural and functional characterization. The glutathione S-transferase (GST) sequence is one of the most commonly used tags for affinity purification of recombinant proteins. However, the 26 kD GST tag is immunogenic and may interfere with the structures and/or biological activities of target proteins, thus necessitating its removal. Moreover, problems such as copurification of proteins interacting with the GST domain or poor efficiency and specificity of the protease towards particular clones demand that the entire protein expression and purification process be monitored. Conventional electrophoretic methods, such as SDS-PAGE, are generally employed for these purposes. We demonstrate that the microfluidics-based Experion automated electrophoresis system, in combination with the Experion Pro260 analysis kit, for the rapid (30 min vs 2–3 hrs), efficient, and effective monitoring of the expression and purification of GST-tagged proteins from crude *E. coli* lysates.

Results

Monitoring Expression Levels Following IPTG Induction

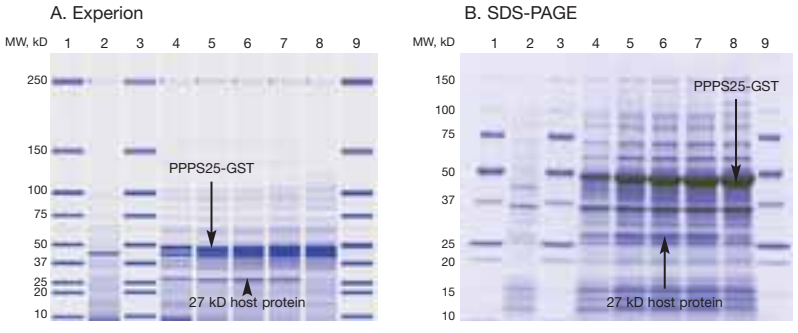

Fig. 1. Expression of a proprietary tagged protein, PPPS25-GST, in *E. coli* BL21(DE3) cell cultures following induction by IPTG. A, simulated gel image generated by the Experion system, showing separation of the Pro260 protein ladder in lanes 1, 3, and 9; B, SDS-PAGE analysis, showing separation of Precision Plus Protein™ standards in lanes 1, 3, and 9. In both images, lane 2 contains sample taken upon IPTG induction; lanes 4–8, samples taken at 1, 2, 3, 4, or 20 hr after induction.

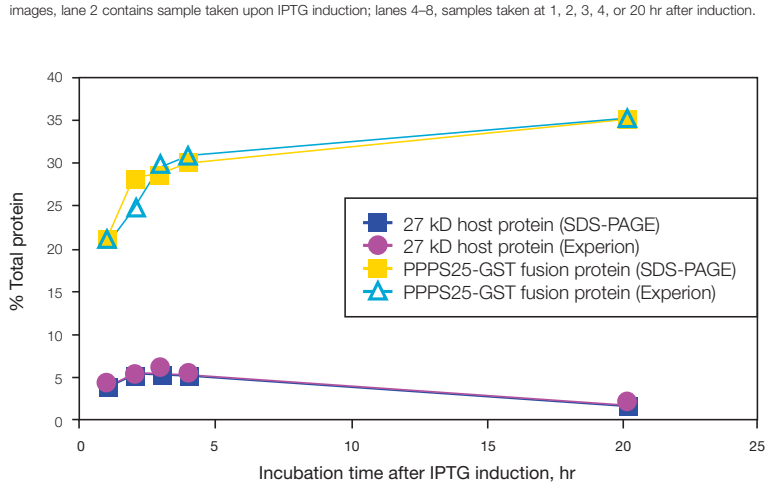

Fig. 2. Abundance of PPPS25-GST fusion protein and a 27 kD host protein in *E. coli* BL21(DE3) cells following induction by IPTG. The expression levels of PPPS25-GST and a 27 kD host protein at various time points were monitored with the Experion system or by SDS-PAGE. With the Experion system, quantitation data are automatically provided.

Monitoring Digestion With Biotinylated Thrombin

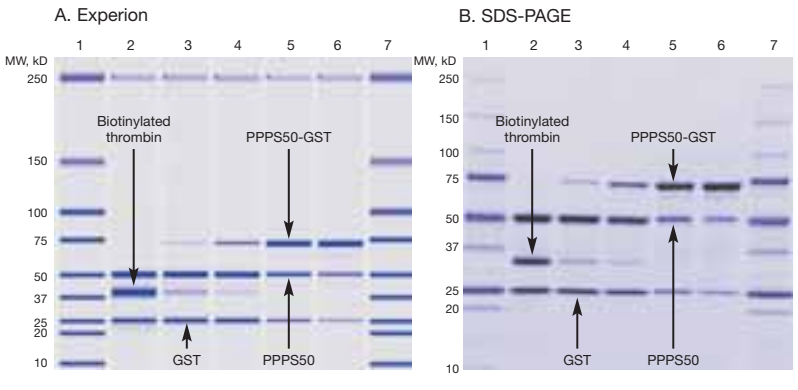

Fig. 3. Cleavage of a proprietary tagged protein, PPPS50-GST, by biotinylated thrombin. A, simulated gel image generated by the Experion system showing separation of the Pro260 protein ladder in lanes 1 and 7; B, SDS-PAGE analysis, showing separation of Precision Plus Protein standards in lanes 1 and 7. In both images, lanes 2–6 show the products from cleavage of 64 µg PPPS50-GST by 1, 0.2, 0.1, 0.02, or 0.01 U biotinylated thrombin after incubation at room temperature for 2 hr.

Monitoring Digestion With Biotinylated Thrombin (cont.)

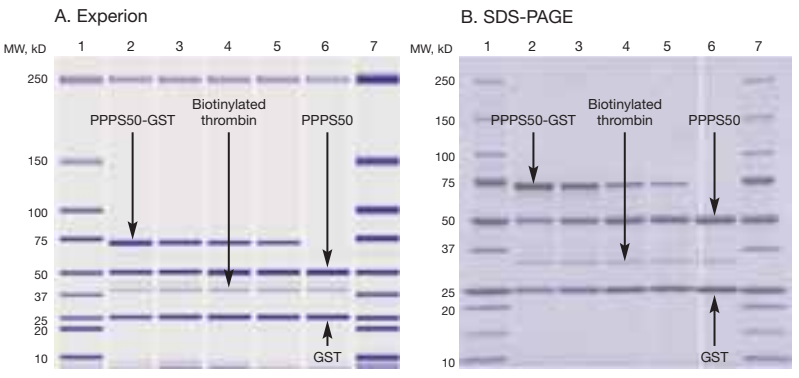

Fig. 4. Time dependence of PPPS50-GST cleavage by biotinylated thrombin. A, simulated gel image generated by the Experion system showing separation of the Pro260 protein ladder in lanes 1 and 7; B, SDS-PAGE analysis, showing separation of Precision Plus Protein standards in lanes 1 and 7. In both images, lanes 2–6 contain samples taken at 0.5, 1, 2, 3, or 20 hr of incubation at room temperature.

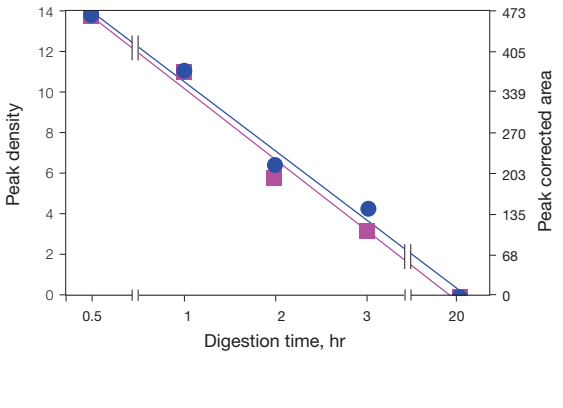

Fig. 5. Kinetics of PPPS50-GST cleavage by biotinylated thrombin at room temperature. Corrected peak area (▬) calculated by Experion software and peak density (▬) detected by Quantity One® software for PPPS50-GST were plotted against digestion time and fitted with linear equations. Coefficients of determination (r^2) for both data sets were 0.99.

Monitoring Purification of Tag-Free Protein

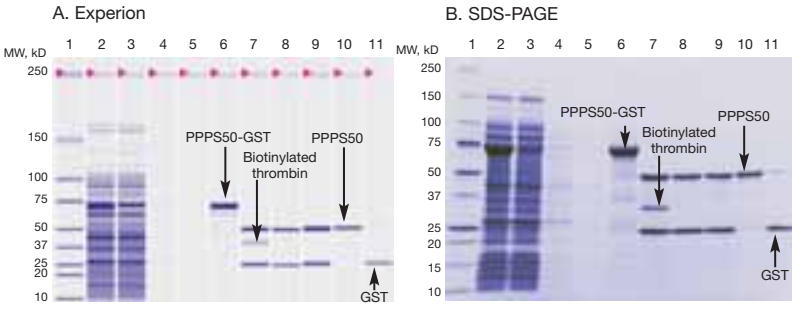

Fig. 6. Purification and processing of PPPS50-GST. A, simulated gel image generated by the Experion system, showing separation of the Pro260 protein ladder in lane 1; B, SDS-PAGE analysis, showing separation of Precision Plus Protein standards in lane 1. In both images, lane 2 contains a separation of crude *E. coli* extract; lane 3, flow-through from a GST MicroSpin column (GE Healthcare); lanes 4 and 5, fractions of unbound proteins washed from the column; lane 6, PPPS50-GST eluate; lane 7, products from biotinylated thrombin digestion of PPPS50-GST; lane 8, sample after biotinylated thrombin removal with streptavidin agarose; lane 9, sample after removal of reduced glutathione with Micro Bio-Spin™ 6 columns; lane 10, tag-free PPPS50 collected in the flow-through from a GST MicroSpin column; and lane 11, GST tag captured by a GST MicroSpin column.

Table 1. Purification analysis.

	Experion		SDS-PAGE	
Sample	Purity	Fold Purification	Purity	Fold Purification
Crude extract	14.0%	1.0	18.4%	1.0
PPPS50-GST	84.9%	6.1	86.6%	4.7
PPPS50	95.7%	6.8	97.3%	5.3

Conclusions

The Experion automated electrophoresis system:

- Integrates protein separation, detection, and analysis
- Reduces reagent and hands-on time required for analysis
- Offers convenience and throughput in protein analysis
- Is an effective tool for protein purification method development and production quality control

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