Rapid and sensitive determination of recombinant protein expression

Introduction

Recombinant protein expression and purification is a multistep process that includes:

- Cloning (transfer of DNA to the host organism by transformation in the case of bacteria, or transfection or transduction in the case of mammalian cells)
- Lysis of cells to recover intracellular proteins, or collection of cell supernatant for secreted proteins
- Selective isolation and purification of the expressed target proteins

Epitope tags are commonly used to allow for easy detection and/or rapid purification of recombinant proteins. These tags are fused to the gene of interest and expressed with the recombinant protein at either the N terminus or C terminus.

Each step of the protein expression and purification process can be very time-consuming; the entire procedure can take several days to weeks for completion. Although there are several stopping points along the way to ensure that the protein of interest is being expressed properly, traditional tests for confirmation of expression (e.g., gel electrophoresis, western blot, ELISA, etc.) can take from several hours to days to complete, adding to the length of the procedure. Thermo Scientific™ Pro-Detect™ Rapid assay kits enable efficient and rapid evaluation of the success of various steps in the protein expression and purification workflow, using common epitopes and fusion tags (Table 1). Pro-Detect Rapid assay kits are single-use, dipstick-style lateral flow strips that provide qualitative confirmation of expressed fusion-tag proteins in 10–15 minutes. These rapid test kits can detect a specific protein tag of interest directly from cell culture media, cell lysate, or purified proteins, and display the result visually without the need for specialized equipment (Figure 1).

Table 1. Epitopes recognized by Pro-Detect Rapid assays, and assay types.

Epitope	Assay type (Cat. No.)
DYKDDDDK (FLAG)	Competitive (A38508)
His (polyhistidine)	Competitive (A38507)
DYKDDDDK and His (double-tagged protein)	Sandwich (A38500)
SUMO (small ubiquitin-like modifier)	Sandwich (A38501)
GST (glutathione S transferase)	Sandwich (A38502)
MBP (maltose binding protein)	Sandwich (A38503)
Human Fc (IgG Fc fragment)	Sandwich (A38504)
Rabbit Fc (IgG Fc fragment)	Sandwich (A38505)
Mouse Fc (IgG Fc fragment)	Sandwich (A38506)
HA (YPYDVPDYA)	Competitive (A38509)
Streptag II (PQKWSHPQFEK)	Competitive (A38510)
AVI (GLNDIFEAQKIEWHE)	Competitive (A38511)
Myc (EQKLISEEDL)	Competitive (A38512)
V5 (GKPIPNPLLGLDST)	Competitive (A38513)

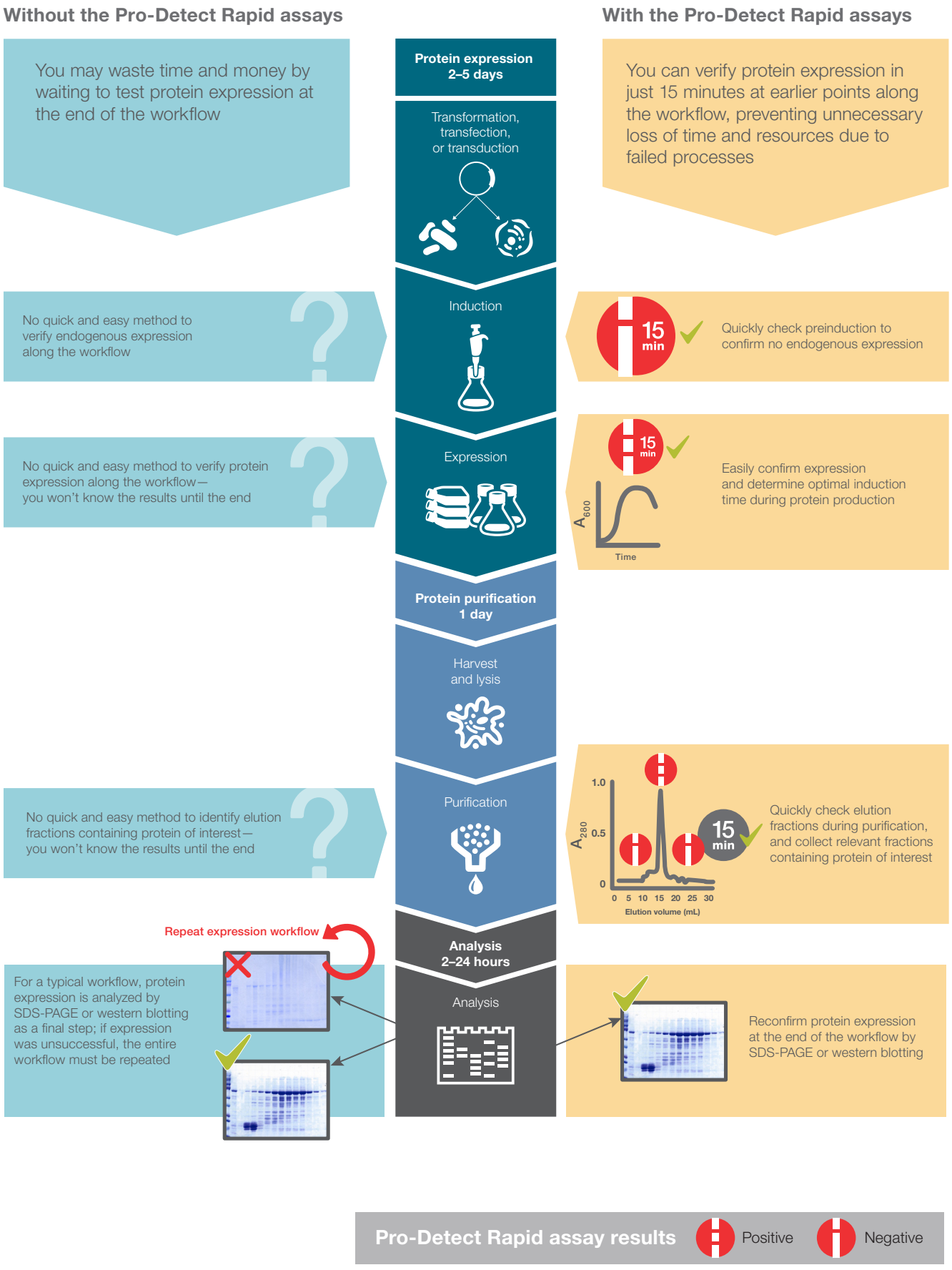

Figure 1. Pro-Detect Rapid assays can be used to monitor the expression and purification of epitope-tagged proteins throughout recombinant protein purification procedures.

Methodology

Pro-Detect Rapid assays are either competitive or sandwich lateral flow assays designed to detect commonly used epitope tags (Table 1). In the sandwich lateral flow assay, a gold-conjugated capture antibody specific to an epitope/analyte is embedded in the pad at the bottom of the strip (Figure 2A). A detection antibody specific to the same or a different epitope of the analyte is immobilized in one test line, and a control antibody, which can also be recognized by the capture antibody, is immobilized in a second line. Upon application of a sample to the strip, the capture antibody binds to the epitope present in the sample while flowing across the membrane; an antibody sandwich is formed at the test line, producing a red line for a positive result. If the test line does not appear, then the sample is negative for the analyte (Figure 2B). The visible appearance of both the test line and the control line indicates the presence of an epitope-tagged protein in the sample (Figure 2C).

In the competitive lateral flow assay, the target epitope/analyte is immobilized on the membrane strip in a group of three test lines. A control antibody, which also can be recognized by the capture antibody, is also immobilized in a distinct single line. A gold-conjugated capture antibody specific to the epitope is embedded in the pad at the bottom of the strip (Figure 3A). If no epitope-tagged protein is present in the sample at a detectable level, the capture antibody will bind to the analyte embedded in the strip and form three red lines, representing a negative result (Figure 3B). If the sample contains protein tagged with the epitope, the capture antibody will bind to the available epitopes in the sample and not to the immobilized epitope target further up the strip (Figure 3C). As the concentration of the tag in the protein sample increases, the number of lines will decrease until they all disappear. The concentration of the tagged proteins is inversely related to the number of lines appearing on the strip.

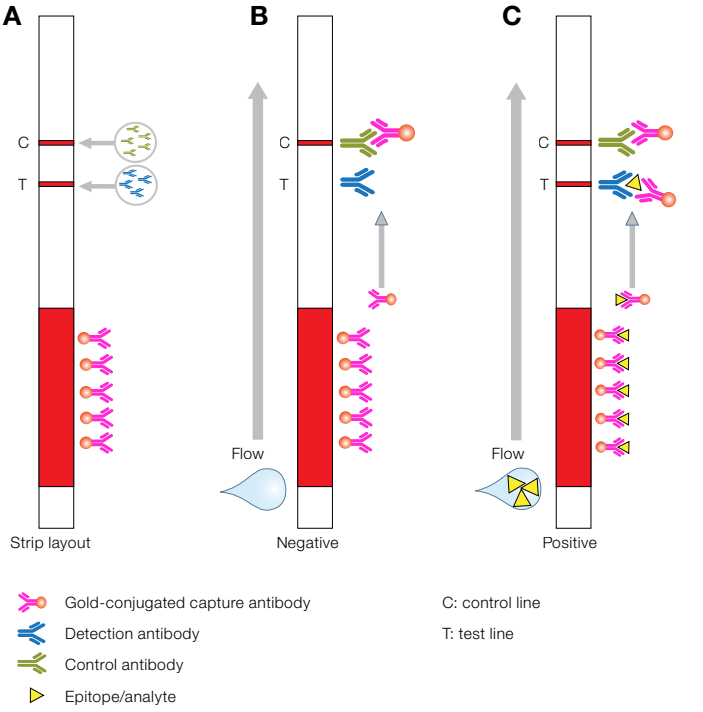

Figure 2. Pro-Detect Rapid sandwich lateral flow assay methodology.

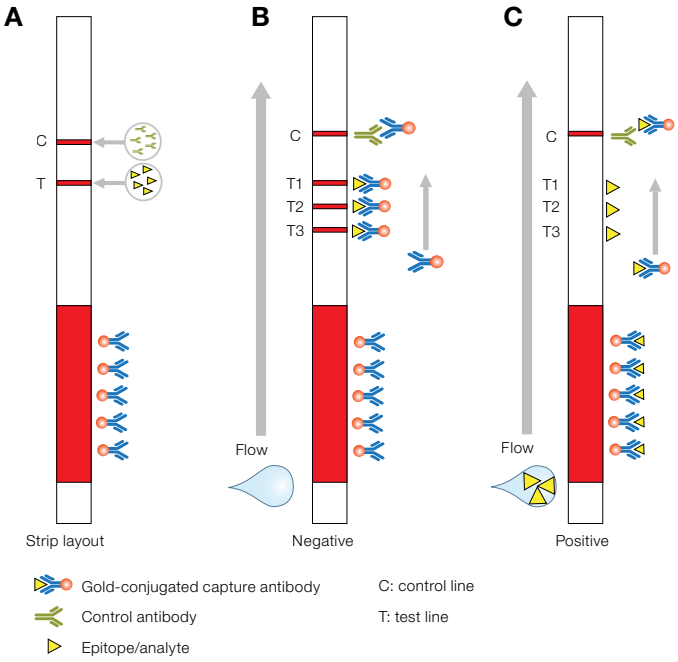

Figure 3. Pro-Detect Rapid competitive lateral flow assay methodology.

Table 2. Detection levels of Pro-Detect Rapid assays.

Assay type	Epitope tag	Recommended range of detection*
Sandwich	DYKDDDDK-His	0.1–10 µg/mL
	SUMO	
	GST	
	MBP	
	Human Fc	
	Rabbit Fc	
	Mouse Fc	
Competitive	His	4–20 µg/mL
	DYKDDDDK	
	HA	
	Streptag II	
	AVI	
	Myc	
	V5	

* Proper sample dilution is essential for optimal results. Concentration ranges are based on the concentration of the tagged protein of interest in the sample.

Sensitivity

Pro-Detect Rapid assays can detect proteins in cell culture media, cell lysates, or purified protein samples, down to the ng/mL level. Each Pro-Detect Rapid assay has been tested for optimal detection using epitope-tagged proteins in lysates as well as in purified protein samples. Pro-Detect Rapid sandwich lateral flow assays have a recommended detection range of 0.1–10 µg/mL (Table 2). These assays can detect outside this range, but the intensity of the red bands is less than desired. Protein concentrations below 0.01 µg/mL were found to be outside the detection range of the strips. Pro-Detect Rapid competitive lateral flow assays have an ideal range of detection of 4–20 µg/mL (Table 2). The assays are able to detect as low as 1 µg/mL, but loss of band intensity (where in this assay format, the presence of the analyte is detected by a loss of color) is less distinguishable. The strips show the maximal "positive" result (disappearance of bands) at analyte concentrations above 20 µg/mL.

Detergent and salt compatibility limits

We examined the performance of Pro-Detect Rapid assays in the presence of various concentrations of detergents and salts that are commonly used in protein expression and isolation. Table 3 summarizes each assay's approximate tolerance level for the tested detergents and salts. Concentrations listed are the actual concentration in the protein sample. The Pro-Detect Rapid assay strips were tested with 0 µg/mL (negative control) or 0.1 µg/mL of a positive-control protein for the sandwich lateral flow assays, and 10 µg/mL of a positive-control protein for the competitive lateral flow assays. All detergents and salts were added to PBS.

Monitoring epitope-tagged proteins during protein expression and purification

The Pro-Detect Rapid assay strips can be used to verify the presence of tagged protein at various stages of protein expression and purification. These steps include protein detection in both uninduced and induced cultures pre- and post-lysis, as well as sequential steps of isolation of purified protein. The Pro-Detect Rapid GST Assay Kit was used to monitor the induction and purification of GST from bacterial cells (Figure 4). GST was cloned into the plasmid pET28a under the control of the *lac* promoter and transformed

Table 3. Detergent and salt compatibility limits.

Assay type	Epitope	NaCl	Urea	Triton X-100	SDS	NP-40	EDTA	Glycerol	KCl	CHAPS	RIPA
Sandwich	DYKDDDDK-His	1.5 M	0.4 M	1%	0.2%	1%	7.5 mM	10%	1.5 M	1%	90%
	SUMO										
	GST										
	MBP										
	Human Fc										
	Rabbit Fc										
	Mouse Fc										
Competitive	His	0.5 M	0.4 M	1%	0.2%	1%	5 mM	10%	0.5 M	1%	90%
	DYKDDDDK	0.25 M	0.4 M	1%	0.2%	1%	5 mM	10%	0.25 M	1%	90%
	HA	0.5 M	0.4 M	1%	0.2%	1%	5 mM	10%	0.5 M	1%	90%
	Streptag II	0.5 M	0.4 M	1%	0.2%	1%	5 mM	10%	0.5 M	1%	90%
	AVI	0.5 M	0.4 M	1%	0.2%	1%	5 mM	10%	0.5 M	1%	90%
	Myc	0.25 M	0.4 M	1%	0.2%	1%	7.5 mM	10%	0.25 M	1%	90%
	V5	1.5 M	0.4 M	1%	0.2%	1%	7.5 mM	10%	1.5 M	1%	90%

into the bacterial cell line, BL21. Protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), and then monitored by analyzing every 30 minutes with the Pro-Detect Rapid GST Assay Kit and absorbance at 600 nm (Figure 4A). Protein expression was monitored by collecting 100 µL of cells at each time point, lysing them in Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent (Cat. 89822), and diluting 50-fold with 1X PBS. The Pro-Detect Rapid GST assay strip was incubated in the diluted lysate for 10 minutes in a 96-well plate. GST was detected as early as 1.5 hours postinduction, and for several hours (Figure 4A). For protein purification, cells

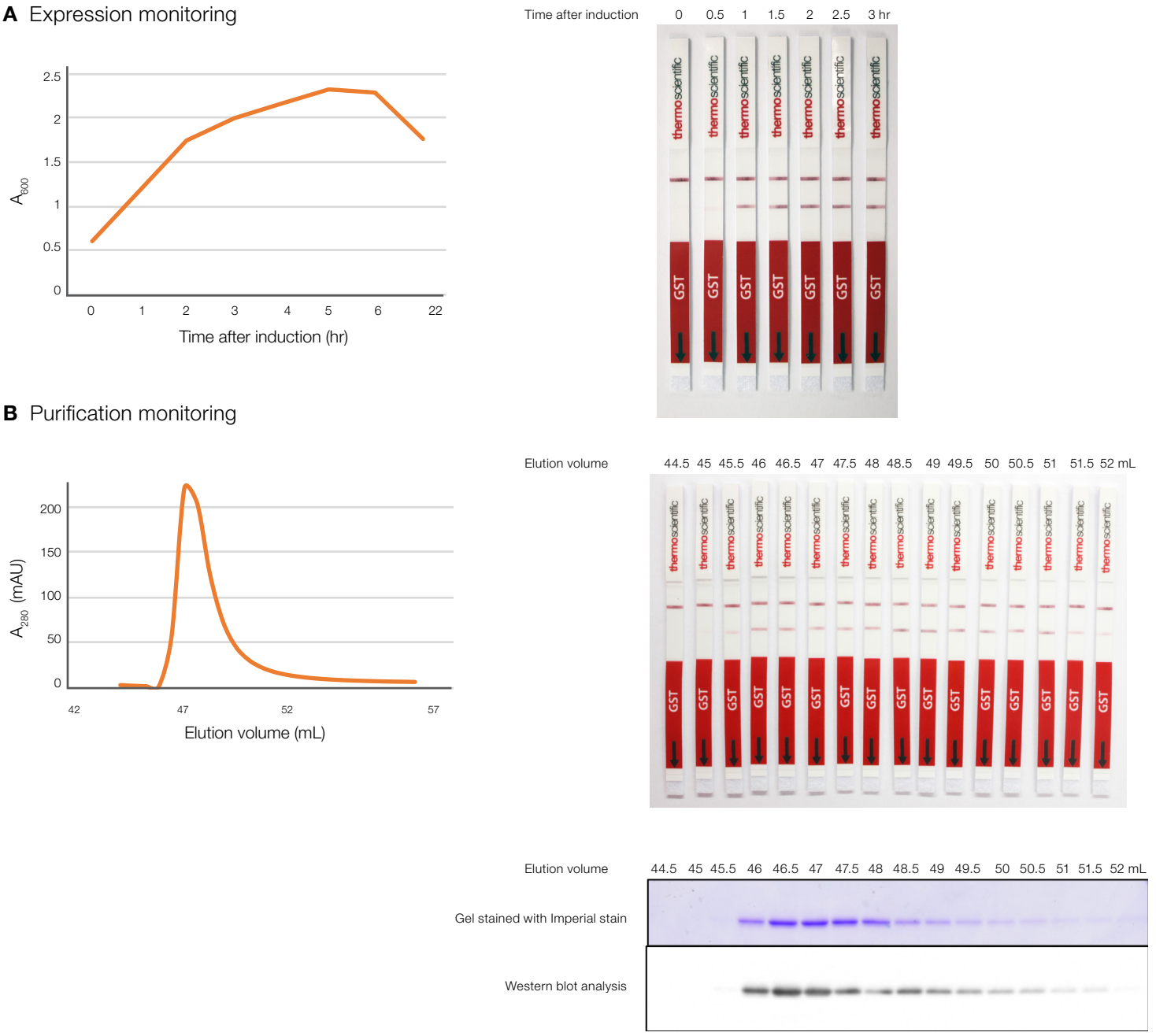

Figure 4. Monitoring expression and purification of GST-GFP using the Pro-Detect Rapid GST Assay Kit.

were collected at 3 hours postinduction and lysed in B-PER buffer according to the product protocol. Protein was purified using Thermo Scientific™ Pierce™ Glutathione Agarose resin in fast protein liquid chromatography (FPLC). Fractions were collected and immediately analyzed using the Pro-Detect strips, as well as by protein electrophoresis and western blotting (Figure 4B). The strips identified GST-containing fractions to be pooled and stored for further purification steps.

Conclusion

The Pro-Detect Rapid assays are quick, easy, and sensitive assays that can be used to analyze the progress of protein expression and purification. The Pro-Detect Rapid assays can facilitate decision-making (i.e., whether to go forward or re-optimize) at each stage, potentially saving hours to days. These assays are compatible with many of the common components in lysis solutions and buffers used to purify proteins from various cell types, including bacterial, yeast, cultured mammalian, and insect cells.

Ordering information

Product	Quantity	Cat. No.
Pro-Detect Rapid DYKDDDDK-His Assay Kit	10 assays	A38500
Pro-Detect Rapid SUMO Assay Kit	10 assays	A38501
Pro-Detect Rapid GST Assay Kit	10 assays	A38502
Pro-Detect Rapid MBP Assay Kit	10 assays	A38503
Pro-Detect Rapid Human Fc Assay Kit	10 assays	A38504
Pro-Detect Rapid Rabbit Fc Assay Kit	10 assays	A38505
Pro-Detect Rapid Mouse Fc Assay Kit	10 assays	A38506
Pro-Detect Rapid His Competitive Assay Kit	10 assays	A38507
Pro-Detect Rapid DYKDDDDK Competitive Assay Kit	10 assays	A38508
Pro-Detect Rapid HA Competitive Assay Kit	10 assays	A38509
Pro-Detect Rapid Streptag II Competitive Assay Kit	10 assays	A38510
Pro-Detect Rapid AVI Competitive Assay Kit	10 assays	A38511
Pro-Detect Rapid Myc Competitive Assay Kit	10 assays	A38512
Pro-Detect Rapid V5 Competitive Assay Kit	10 assays	A38513

Learn more at **thermofisher.com/prodetect**

ThermoFisher SCIENTIFIC