The S30 T7 High-Yield Protein Expression System

ABSTRACT The S30 T7 High-Yield Protein Expression System is an *E. coli* extract-based cell-free protein synthesis system that transcribes and translates DNA sequences cloned in vectors containing a T7 promoter or a strong *E. coli* promoter. Protein yield can reach hundreds of micrograms per milliliter of reaction (up to 500 µg/ml) within 1 hour at 37 °C. The reaction volume can be scaled down to as little as 5 µl for high-throughput applications.

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Proteins expressed in the T7 S30 High-Yield System are more stable than those expressed in vivo, because the *E. coli* extract is deficient in OmpT and lon proteases.

INTRODUCTION

The S30 T7 High-Yield Protein Expression System[a] is an *E. coli* extract-based cell-free protein synthesis system. This system simplifies the transcription and translation of DNA sequences cloned in vectors containing a T7 promoter by providing an extract that contains T7 RNA polymerase for transcription and all necessary components for translation (Figure 1).

The extract is prepared from an *E. coli* strain B deficient in OmpT endoproteinase and lon protease activity. This results in greater stability of expressed proteins that otherwise would be degraded by proteases. An optimized premix provides all other required components, including amino acids, rNTPs, tRNAs, an ATP-regenerating system, IPTG and appropriate salts. The system expresses high levels of recombinant proteins (up to hundreds of micrograms of recombinant protein per milliliter of reaction) within an hour. The investigator supplies only cloned DNA containing a protein-coding region downstream of a T7 promoter and a ribosome-binding site (RBS).

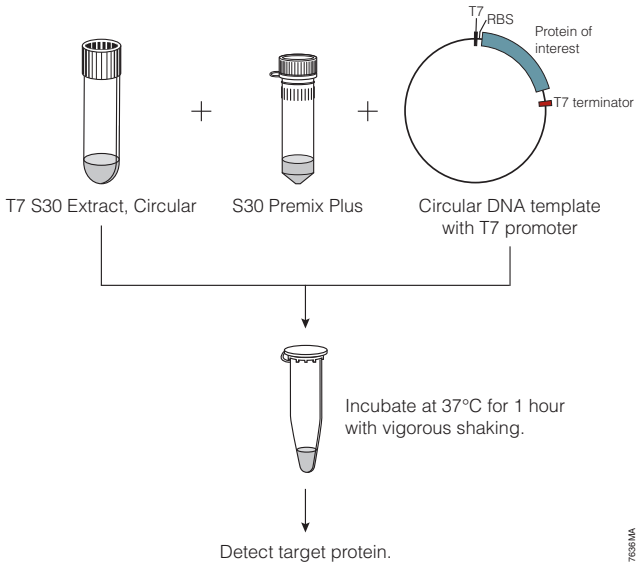

Figure 1. Schematic for the S30 T7 High-Yield Protein Expression System.

DETECTION METHODS

After protein synthesis, several detection methods can be used. The proteins generated at high levels (up to 500 µg/ml) with the S30 T7 High-Yield Protein Expression System can be detected by Coomassie® blue-stained SDS-PAGE (Figure 2, Panel A). Synthesized proteins also can be detected by two non-radioactive detection methods: the FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (Cat.# L5001), which fluorescently labels proteins synthesized in vitro, or the Transcend™ Non-Radioactive Translation Detection System (Cat.# L5070, L5080), which biotinylates the target protein (Figure 2, Panels B and C).

TEMPLATE CONSIDERATIONS

Expression of cloned DNA fragments in the S30 T7 High-Yield Protein Expression System requires a protein-coding sequence under the control of either a T7 promoter or a strong *E. coli* promoter in a circular vector. Successfully tested vectors are shown in Table 1. Expression from T7 promoters is typically higher than from *E. coli* promoters using this system.

Table 1. Vectors Used for Successful Protein Synthesis in the S30 T7 High-Yield Protein Expression System. (Listed proteins were detected by SDS-PAGE and Coomassie® blue staining.)

Vector	Promoter	Proteins Tested
pFN6A/K	T7	firefly and *Renilla* luciferase, HaloTag® protein, Monster Green® fluorescent protein, cPKA, β-galactosidase
pFN18A/K	T7	cPKA, firefly and *Renilla* luciferase, Id
pIVEX	T7	elongation factor Ts (EF-Ts)
pIX	T7	green fluorescent protein (GFP)
pExp5	T7	calmodulin3 (CALM3)
pET32a	T7	Thioredoxin
pET43a	T7	NusA
pET3a	T7	*Renilla* luciferase
pET15b	T7	*Renilla* luciferase
pQE30	T5	firefly and *Renilla* luciferase

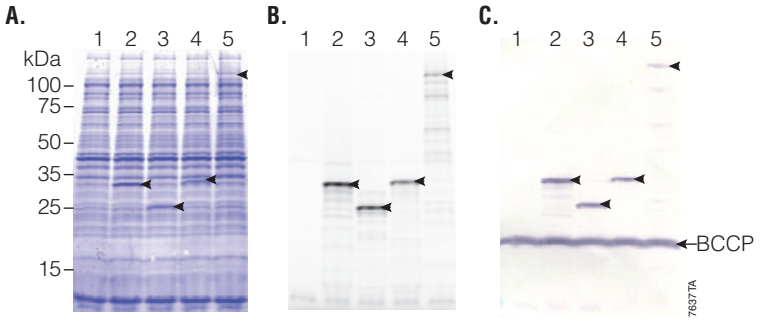

Figure 2. Coupled in vitro transcription/translation of circular DNA templates using the S30 T7 High-Yield Protein Expression System. The protein-coding sequences cloned into pFN6A (HQ) Flexi® Vector were expressed as described in the *S30 T7 High-Yield Protein Expression System Technical Manual* #TM306, resolved by SDS-polyacrylamide gel electrophoresis (PAGE; 4–20% Tris-glycine) and visualized by Coomassie® blue staining (**Panel A**), fluorescent scanning (**Panel B**), or transferred to PVDF membrane, treated with Streptavidin Alkaline Phosphatase (Cat.# V5591) and stained with Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841; **Panel C**). For each gel: lane 1, no DNA; lane 2, *Renilla* luciferase; lane 3, Monster Green® Fluorescent Protein; lane 4, HaloTag® protein; lane 5, β-galactosidase. (BCCP = *E. coli* biotin carboxyl carrier protein.)

Detect proteins expressed with the cell-free system using Coomassie® blue staining, fluorescent labeling or biotinylation.

Large differences in gene expression can be the result of the size of the protein, the gene of interest itself, the context in which the gene resides, the expression conditions used, and changes in the position of the protein-coding sequence relative to the RBS (1). In addition, many eukaryotic genes contain sequences within the protein-coding region that can function as RBSs when they precede a methionine codon, which can result in internal translation initiation and the synthesis of potentially undesired truncated proteins in the prokaryotic system.

Other factors have been reported to affect protein synthesis in S30 systems, including 5′ and 3′ untranslated regions (UTRs; 2,3), N- (4) or C-terminal (5) fusion tags, codon usage (6), mRNA secondary structure (7,8) and mRNA stability (9).

Highly purified plasmid DNA templates with concentrations at 500 ng/µl or higher will achieve the best protein yield. We recommend purifying template DNA with the PureYield™ Plasmid Miniprep System (Cat.# A1221) for use with this transcription/translation system. Avoid adding high concentrations of salts or glycerol with the DNA template. Ethanol precipitate the DNA template with sodium acetate rather than ammonium acetate. Typical protein yields can reach tens of micrograms per 50 µl reaction (up to 500 µg/ml).

APPLICATIONS

- verify the gene product of a particular DNA sequence
- screen proteins in high-throughput applications (e.g., in 96-well plates with volumes as low as 5 µl)
- produce proteins toxic to *E. coli* cells
- purify using affinity tags (e.g., metal-affinity tag)
- analyze enzyme activity
- study protein interactions
- examine transcription and translation (10)
- incorporate unnatural amino acids into proteins (11)
- screen compounds that affect translation (12)

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PROTOCOL

- *S30 T7 High-Yield Protein Expression System Technical Manual* #TM306, Promega Corporation **www.promega.com/tm306/tm306.html**

ORDERING INFORMATION

Product	Size	Cat.#
S30 T7 High-Yield Protein Expression System	24 reactions	L1110
	8 reactions	L1115
FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System*	40 reactions	L5001
Transcend™ Colorimetric Non-Radioactive Translation Detection System*	30 reactions	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System*	30 reactions	L5080
PureYield™ Plasmid Miniprep System	50 preps	A1221
	250 preps	A1222

*For Laboratory Use.