Higher Yields Plus Single-Tube Format

Wheat Germ Extract Plus For Higher Protein Yields

By Robin Hurst, M.S., Elaine Schenborn, Ph.D., John Shultz, Ph.D., Sanchayita Kar, Ph.D., Donna Leippe, Ph.D., and Don Creswell, B.S., Promega Corporation

Abstract

This article compares the yields from Wheat Germ Extract Plus and our conventional Wheat Germ Extract using various transcripts encoding multiple proteins. Up to 22-fold higher yields were achieved using the Wheat Germ Extract Plus. Two new Wheat Germ Flexi® Vectors are also available to make optimized template mRNA.

Wheat Germ Extract Plus uses a single-tube format to express proteins in the range of 10–80µg/ml.

Introduction

For decades wheat germ extracts have been used for eukaryotic cell-free translation, demonstrating good fidelity while being hampered by low efficiency of translation (1–3). We have developed a cost-effective, robust, high-efficiency Wheat Germ Extract Plus that uses a single-tube format to express proteins in the range of 10–80µg/ml.

Wheat Germ Extract Plus is optimized for many types of mRNA templates: uncapped, capped or with a viral leader at the 5′ end and a poly(A) tail or viral translation enhancers at the 3′ end. A poly(A) tail or 3′ viral translation enhancer significantly increases the translation efficiency in Wheat Germ Extract Plus, possibly due to the formation of an mRNA loop (4).

The Wheat Germ Flexi® Vectors[a,b] (Cat.# L5671 and L5681) shown in Figure 1 can be used to incorporate 5′ UTR leader and 3′ TE (translation enhancer) regions from the barley yellow dwarf virus (BYDV) into mRNA generated in vitro for translation applications. The BYDV elements reportedly interact to form a closed loop and act synergistically to stimulate translation in wheat germ extracts, bypassing mRNA cap and polyadenylation dependencies (5–8). When using the Flexi® Wheat Germ Vectors either T7 or SP6 RNA polymerase can be used to generate mRNA, resulting in equivalent protein production. Linear or circular DNA plasmids can be used as templates for transcription.

In this article we compare the translation efficiency of the new Wheat Germ Extract Plus to the conventional Wheat Germ Extract (Cat.# L4380) using transcripts encoding five different proteins.

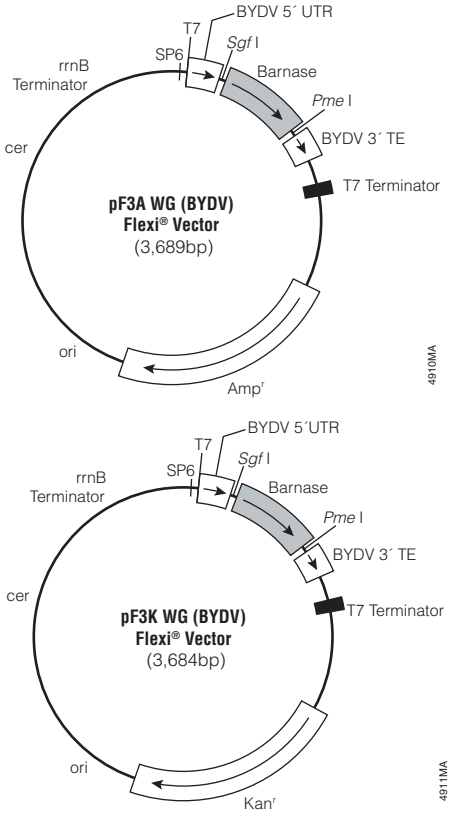

Figure 1. Features of the Wheat Germ Flexi® Vectors. The pF3A WG (BYDV) and pF3K WG (BYDV) Flexi® Vectors (Cat.# L5671 and L5681, respectively) are designed for expression of proteins in wheat germ extract. These vectors incorporate sequences from barley yellow dwarf virus (BYDV) upstream and downstream of the protein coding region of interest. The vectors contain *Sgf* I and *Pme* I sites to facilitate directional cloning and transfer of protein-coding sequences to other Flexi® Vectors with different expression options. The lethal barnase gene allows positive selection of vectors containing insert. Ampicillin [pF3A WG (BYDV) Flexi® Vector] and kanamycin [pF3K WG (BYDV) Flexi® Vector] resistance genes allow selection in *E. coli*. Please refer to the *Flexi® Vector Systems Technical Manual* #TM254 for further details on the Flexi® Vector technology.

Comparison of Translation Efficiency and Protein Expression

Wheat Germ Extract Plus is an optimized extract that contains all the necessary components for efficient translation for a wide range of mRNA templates. For the following experiments, RNAs with various 5′ and 3′ sequence features, coding for different proteins, were synthesized in vitro and purified (Table 1). Transcripts were generated from linear plasmid templates using T7 RiboMAX™ Express Large Scale RNA Production System (Cat.# P1320) followed by gel filtration cleanup.

Table 1. Transcript and Expressed Protein Properties.

Name	Protein	Molecular Weight	Source	Translation Elements
poly(A) luciferase	firefly luciferase	61kDa	Luciferase Control RNA (Cat.# L4561)	poly(A) tail; no 5′ cap
BYDV luciferase	firefly luciferase	61kDa	pF3K WG (BYDV) Flexi® Vector clone	5′ and 3′ BYDV enhancers
poly(A) *Renilla*	*Renilla* luciferase	36kDa	pLVSK clone (pF3K precursor)	poly(A) tail; no 5′ cap
BYDV *Renilla*	*Renilla* luciferase	36kDa	pF3K WG (BYDV) Flexi® Vector clone	5′ and 3′ BYDV enhancers
poly(A) GST	glutathione S-transferase	26kDa	pLVSK clone (pF3K precursor)	poly(A) tail; no 5′ cap
BYDV GST	glutathione S-transferase	26kDa	pF3K WG (BYDV) Flexi® Vector clone	5′ and 3′ BYDV enhancers
poly(A) HaloTag™	haloalkane dehalogenase	33kDa	pLVSK clone (pF3K precursor)	poly(A) tail; no 5′ cap
BYDV HaloTag™	haloalkane dehalogenase	33kDa	pF3K WG (BYDV) Flexi® Vector clone	5′ and 3′ BYDV enhancers
BYDV β-gal	β-galactosidase	135kDa	pF3A WG (BYDV) Flexi® Vector clone	5′ and 3′ BYDV enhancers

The β-gal RNA, was derived from circular plasmid DNA. We recommend using 0.12–0.16µg/µl of transcript per reaction when performing translation reactions in Wheat Germ Extract Plus.

As shown in Figure 2, the Wheat Germ Extract Plus translation time course is linear for 60 minutes in batch mode using poly(A) luciferase and BYDV luciferase transcripts encoding firefly luciferase (Table 1). The time course experiment was also monitored with [^{35}S]methionine, and the results were identical to the luciferase activity assay study (data not shown).

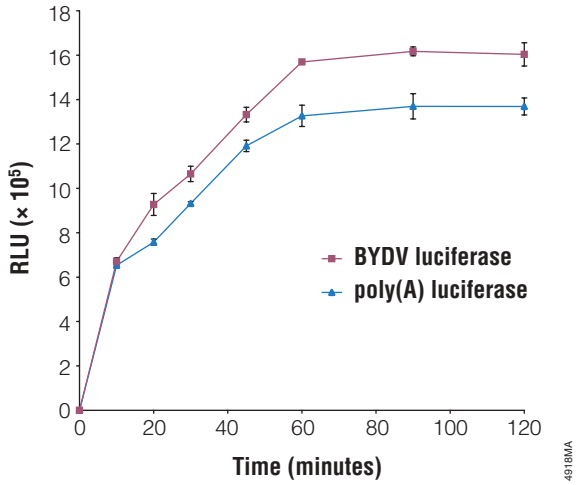

Figure 2. Time course of active firefly luciferase expression in Wheat Germ Extract Plus. Transcripts were purified with NAP™-10 columns and used in reactions at a concentration of 0.16µg/µl. Each reaction was incubated at 25°C for the specified time, then 2.5µl of the reaction was assayed in 50µl of Luciferase Assay Reagent (LAR) in a Turner TD-20/20 Luminometer. Results are expressed as Relative Light Units (RLU) for the mean and standard deviation of triplicate reactions.

Figure 3 compares the levels of protein synthesis as monitored by [^{35}S]methionine incorporation. The reactions shown in Figure 3 used eight different transcripts encoding four different proteins ranging in size from 26-61kDa (Table 1). Quantitation data from the gels indicate that Wheat Germ Extract Plus made 8.5- to 17-fold more protein than the conventional extract. These results also demonstrate that Wheat Germ Extract Plus can be used to monitor protein expression by radioactive methionine incorporation. In addition, Protein expression can be monitored non-radioactively using FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Cat.# L5001) or Transcend™ Non-Radioactive Translation Detection Systems (Cat.# L5070, L5080; data not shown). Figure 3 also illustrates that RNA templates with BYDV sequences can produce higher levels of expressed proteins, and the magnitude of this increase may be protein-dependent.

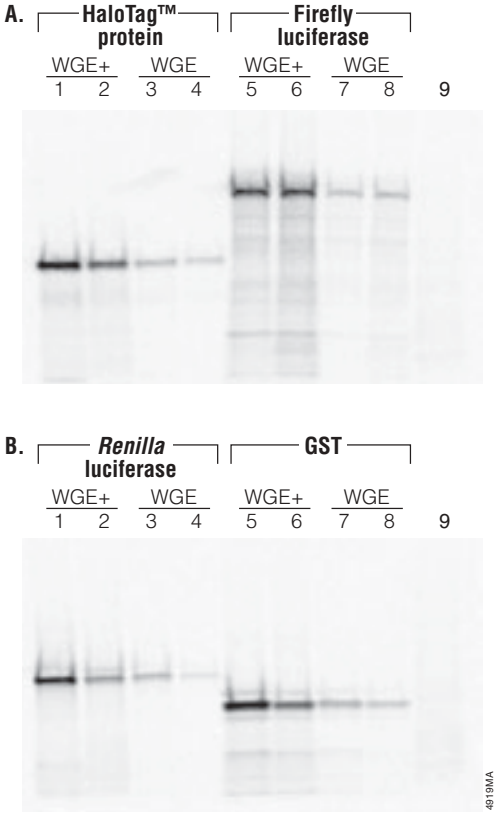

Figure 3. Comparison of Wheat Germ Extract Plus (WGE+) and conventional Wheat Germ Extract (WGE) reactions. All reactions were performed with 1µl of [^{35}S]methionine (>1,000Ci/mmol) and 0.16µg/µl of mRNA. Reactions were incubated for 2 hours at 25°C. One microliter of each reaction was loaded on a 4–20% polyacrylamide gel. Gels were transferred to PVDF and exposed to a phosphorimaging cassette for two hours. The cassette was read and quantitated using the Typhoon™ 9410 workstation. Lanes 1, 2, 5, and 6 are Wheat Germ Extract Plus and lanes 3, 4, 7, and 8 are Wheat Germ Extract. Lane 9 is Wheat Germ Extract Plus without mRNA. **Panel A.** Lanes 1 and 3 use the BYDV HaloTag™ transcript, and lanes 2 and 4 use the poly(A) HaloTag™ transcript. Lanes 5 and 7 use the BYDV luciferase transcript, and lanes 6 and 8 use the poly(A) luciferase transcript. **Panel B.** Lanes 1 and 3 use the BYDV *Renilla* transcript, and lanes 2 and 4 use the poly(A) *Renilla* transcript. Lanes 5 and 7 use the BYDV GST transcript, and lanes 6 and 8 use the poly(A) GST transcript. See Table 1 for a description of transcripts.

Table 2. Comparison of GST and Firefly Luciferase Protein Levels in Wheat Germ Extract Plus and Wheat Germ Extract.

Type of Lysate	BYDV GST	poly(A) GST	BYDV luciferase	poly(A) luciferase
Wheat Germ Extract Plus	82.8µg/ml	24.1µg/ml	56µg/ml	51µg/ml
Wheat Germ Extract	3.7µg/ml	1.5µg/ml	3.5µg/ml	3.5µg/ml

GST protein was quantitated by immunoassay using the GST 96 Well Detection Module (GE Healthcare, Cat.# 27-4592-01). Luciferase was quantitated based on a standard activity curve using QuantiLum® Recombinant Luciferase (Cat.# E1701).

GST and firefly luciferase proteins were also quantitated directly in the lysate by either immunoassay or activity, respectively. The results are summarized in Table 2. Wheat Germ Extract Plus produced approximately 16- to 22-fold more GST and 15-fold more active firefly luciferase protein than the conventional Wheat Germ Extract. Furthermore, RNA transcripts for GST with BYDV sequences produced more than 3-fold more GST protein in Wheat Germ Extract Plus than transcripts with poly(A) tails and no BYDV sequences.

Figure 4 compares the expression of a larger protein in the two wheat germ extracts using varying amounts of template mRNA. Wheat Germ Extract Plus effectively expressed more of the 135kDa β-galactosidase for all template concentrations tested. Quantitation analysis shows that the Wheat Germ Extract Plus expressed six times more full-length β-galactosidase than the conventional extract using 0.16µg/µl template mRNA.

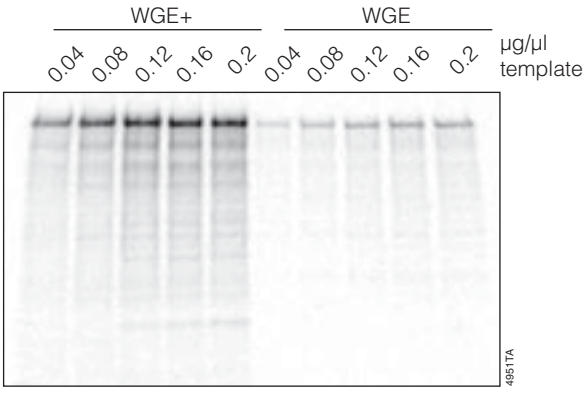

Figure 4. Titration of BYDV β-Galactosidase mRNA in Wheat Germ Extract Plus and in Wheat Germ Extract. The indicated concentrations of NAP™-5-purified mRNA were used in either Wheat Germ Extract Plus (WGE+) or Wheat Germ Extract (WGE) reactions. Protein synthesis levels were monitored with [^{35}S]methionine incorporation as detected on 4–20% polyacrylamide gels by phosphorimaging with the Typhoon™ 9410 workstation.

Summary

Wheat Germ Extract Plus, a eukaryotic cell-free translation system, offers significant advantages over traditional wheat germ extract systems with increased translation activity in batch reactions yielding protein expression levels of 10–80µg/ml. Wheat Germ Extract Plus provides optimized components for efficient translation of a wide variety of RNA templates. The easy-to-use, single-tube format provides everything required for translation except the mRNA template and desired label. Flexi® Vectors with BYDV translation-enhancing sequences are also available for increasing in vitro protein expression.

References

1. Roberts, B.E. and Paterson, B.M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2330–4.
2. Erickson, A.H. and Blobel, G. (1983) *Methods Enzymol.* **96**, 38–50.
3. Seal, S.N., Schmidt, A. and Marcus, A. (1986) *Methods Enzymol.* **118**, 128–40.
4. Gallie, D.R. (1998) *Gene* **216**, 1–11.
5. Wang, S. and Miller, W.A. (1995) *J. Biol. Chem.* **270**, 13446–52.
6. Wang, S., Browning, K.S. and Miller, W.A. (1997) *EMBO J.* **16**, 4107–16.
7. Guo, L., Allen, E.M. and Miller, W.A. (2000) *RNA* **6**, 1808–20.
8. Guo, L., Allen, E.M. and Miller, W.A. (2001) *Mol. Cell* **7**, 1103–9.

Protocols

- *Wheat Germ Extract Plus Technical Manual* #TM066, Promega Corporation. (www.promega.com/tbs/tm066/tm066.html)

Ordering Information

Product	Size	Cat.#
Wheat Germ Extract Plus	40 × 50µl reactions	L3250
	10 × 50µl reactions	L3251
pF3A WG (BYDV) Flexi® Vector	20µg	L5671
pF3K WG (BYDV) Flexi® Vector	20µg	L5681

[a] Patent Pending.

[b] For research use only. Persons wishing to use this product or its derivatives in other fields of use, including without limitation, commercial sale, diagnostics or therapeutics, should contact Promega Corporation for licensing information.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.