



## In Vitro Translation Systems – Protein expression

### ▪ Human In Vitro Translation Kits and Reagents

The 1-Step Human In Vitro Translation System is a method for expressing proteins from DNA or mRNA templates in a cell-free solution containing essential components of the cellular translational machinery. Extracts of an immortalized human cell line provide the ribosomes, initiation and elongation factors, tRNAs and other basic components required for protein synthesis. When supplemented with proprietary accessory proteins, ATP, and an energy regenerating system, these extracts sustain the synthesis of target proteins from DNA templates for up to 6 hours without the need to remove inhibitory byproducts.

#### 1-Step Human Coupled In Vitro Expression Kits

Rapidly express full-length, functional proteins from mRNA or plasmid templates with yields of up to 100µg/mL per reaction using these cell-free kits.

#### 1-Step Human High-Yield In Vitro Expression Kits

Express high concentrations (up to 750 micrograms per milliliter) of functional mammalian protein with these eukaryotic IVT protein expression kits that use integrated continuous feed dialysis devices.

#### 1-Step Heavy Protein IVT Kit

Express stable isotope-labeled (i.e., heavy) proteins by in vitro translation (IVT) for mass spectrometry using these cell-free expression kits.

#### T7 Vectors for Cell-free Protein Expression

Generate His-, HA-, Myc- or other label-tagged fusion proteins by cell-free in vitro translation (IVT) with these versatile T7 expression vectors.



## 1-Step Human In Vitro Protein Expression Kits

*Express full-length functional proteins in 90 minutes.*

1-Step Human *In Vitro* Protein Expression Kits enable the translation and post-transcriptional modification of full-length proteins from mRNA or plasmid templates with yields of up to 100µg/mL per reaction.

The Human IVT Kits are unique HeLa cell lysate-based protein expression systems for *in vitro* translation or coupled transcription/translation reactions. Protein expression is performed in a single 90-minute reaction that can be extended for up to 6 hours with continued protein production up to 100µg/mL when combined with the optimized pT7CFE1 Expression Vector. The Human IVT Kits can express functional enzymes, phosphoproteins, glycoproteins and membrane proteins for immediate use in studying protein interactions, performing rapid mutational analysis and measuring activity.



### Highlights:

- **Functional** – uses the human translational machinery to express active proteins
- **Convenient** – perform transcription and translation in a single step
- **High performance** – greater yields compared to rabbit reticulocyte *in vitro* translation
- **Reliable** – express proteins that fail in rabbit reticulocyte systems

### Better Than Traditional Methods:

- HeLa cell-free extracts are capable of expressing proteins with post-translational modifications
- Accurate translation delivers full-length protein compatible with downstream applications
- Protein translation is optimized with EMCV IRES element and other mRNA stabilizing elements

Includes: Complete kits contain HeLa cell lysate, accessory proteins, reaction mix, nuclease-free water, expression vector and a GFP-positive control vector

Requires: 30°C incubator or water bath

### Applications:

- Express proteins to measure enzyme activity
- Express protein for use in gel mobility shift assays
- Express cytotoxic proteins
- Perform unnatural amino acid labeling
- Perform mutational analysis
- Perform co-immunoprecipitation

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	<a href="#">Price</a>
88881	<b>1-Step Human Coupled IVT Kit - DNA</b>					
88882	<i>Sufficient For: 8 reactions of 25µL each</i>	40-rxn kit				
	Kit Contents (8-rxn kit kit): HeLa Lysate, 110µL Accessory Proteins, 25µL Reaction Mix, 40µL Positive Control DNA: pCFE-GFP, 10µg pT7CFE1-CHis, 10µg Nuclease-Free Water, 1.5mL					



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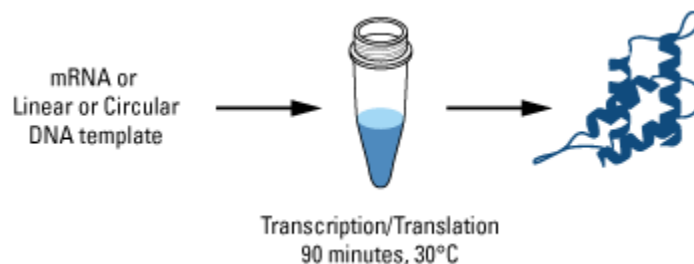
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88880	<b>tGFP mRNA</b> 0.75µg/µL mRNA in 0.1mM EDTA	10µg
88899	<b>Recombinant GFP Protein</b>	20µg



### Product Details:

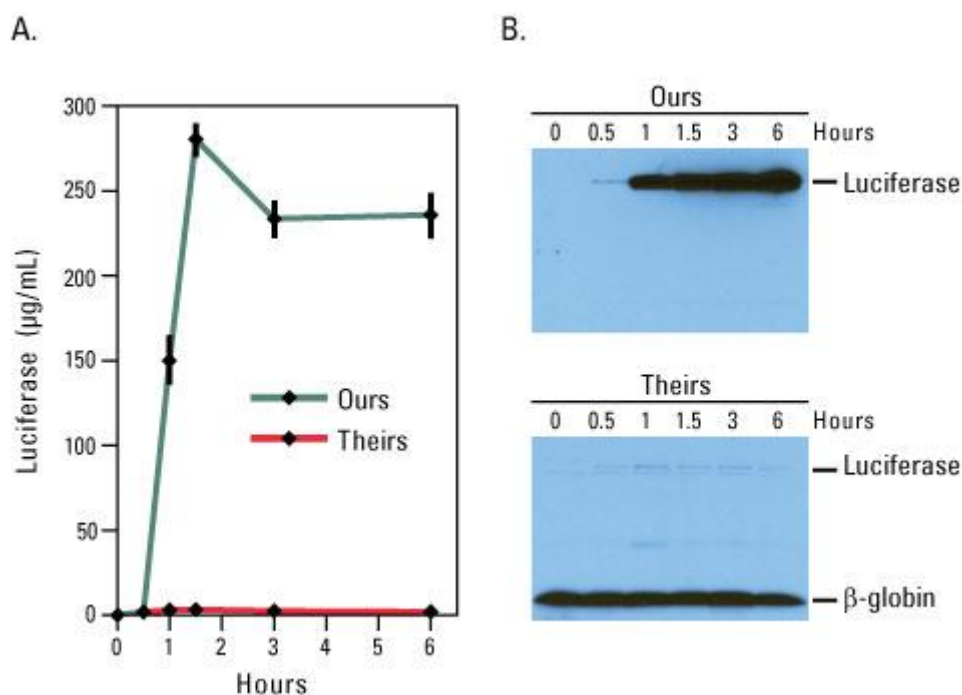


**Schematic of the 1-Step Coupled Human IVT Kit for DNA and 1-Step Human IVT Kit for RNA.** Simply add the appropriate template to a mixture of HeLa cell lysate, Accessory Proteins, Reaction Mix and incubate at 30°C for 90 minutes for protein yields up to 100µg/mL. Smaller reactions are ideal for expression of mutational variants in a microplate format. The reaction volumes and times can be increased to express larger amounts of a single protein for use in several downstream applications.

**Note:** For even more protein expression, 250 to 750µg/mL of protein can be achieved using the 1-Step High-Yield IVT Kits.

The 1-Step Coupled Human IVT Kit for DNA is a cell-free system using the cellular transcription and translation machinery from a modified HeLa cell line. The procedure is quick and easy and is an effective alternative to other protein expression methods. Simply add an appropriate expression construct to a mixture of HeLa cell lysate, Accessory Proteins, Reaction Mix and incubate at 30°C for 90 minutes to overnight.

The 1-Step Human IVT Kit for mRNA also uses the human protein translation machinery to produce functional protein. This kit is recommended for translation of mRNA transcripts containing an EMCV IRES element and mRNA stabilizing features designed into the pT7CFE1 expression vector.



The 1-Step Human Coupled Human IVT Kit produces more active protein without

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**interfering substances.** *In vitro* luciferase expression reactions were performed with the 1-Step Human Coupled IVT Kit and the Promega TnT\* T7 Quick Coupled Transcription/Translation System according to supplied instructions and control plasmids. Samples were removed from each reaction at the indicated intervals and analyzed for (A.) luciferase activity (correlated to µg/mL of active protein) or (B.) Western blot (1µL). The 1-Step Coupled Human In Vitro Expression Kit produced luciferase protein without contaminating beta-globin.

The human *in vitro* translation system is robust and will express proteins from a variety of species including mammals, bacteria and protozoa. Benchmarking shows that the expression levels of functional proteins such as luciferase are much higher in the human system compared to either rabbit reticulocyte-based or *E. coli*-based systems. Furthermore, proteins expressed with the human *in vitro* translation system are not contaminated with substances that can interfere with downstream applications.

#### Product References:

1. Heidary DK, Glazer EC.(2014). A light-activated metal complex targets both DNA and RNA in a fluorescent in Vitro transcription and translation assay. *Chembiochem*. 15(4):507-11.
2. Festa F. et al. (2013) Robust microarray production of freshly expressed proteins in a human milieu. *Proteomics Clin Appl*. 7(5-6):372-7. **Published by Joshua LaBaer's group.**
3. Wang J. et al. (2013) A versatile protein microarray platform enabling antibody profiling against denatured proteins. *Proteomics Clin Appl*. 7(5-6):378-83.
4. Yadavalli, R., Ledger, C., Sam-Yellowe, TY. (2012). In vitro human cell-free expression system for synthesis of malaria proteins. *Parasitol Res*. 111(6):2461-5.
5. Boohaker R. J. et al. (2011). BAX supports the mitochondrial network, promoting bioenergetics in nonapoptotic cells. *Am J Physiol Cell Physiol*. 300, C1466-78.
6. Loughran G. et al. (2011). Ribosomal frameshifting into an overlapping gene in the 2B-encoding region of the cardiovirus genome. *Proc Natl Acad Sci USA*. 108, E1111-9.
7. Stergachis, A. (2011). Rapid empirical discovery of optimal peptides for targeted proteomics. *Nature Methods*, 8:1041-3.
8. Wang Q. Y. et al. (2011). A translation inhibitor that suppresses dengue virus *in vitro* and *in vivo*. *Antimicrob Agents Chemother*. 55:4072-80.
9. Boyne, J. (2010). Kaposi's sarcoma-associated herpesvirus ORF57 protein interacts with PYM to enhance translation of viral intronless mRNAs. *EMBO Journal*, 29:1851-64.
10. Kasinathan, R., (2010). Schistosoma mansoni express higher levels of multidrug resistance-associated protein 1 (SmMRP1) in juvenile worms and in response to praziquantel. *Molecular and Biochemical Parasitology*, 173:25-31.
11. Khatua, A. (2010). Inhibition of LINE-1 and Alu retrotransposition by exosomes encapsidating APOBEC3G and APOBEC3F. *Virology*, 400:68-75.

#### General References:

1. Imataka, H., and Mikami, S. (2009). Advantages of human cell-derived cell-free protein synthesis systems (Japanese). *Seikagaku* 81(4):303-7.
2. Kobayashi, T., et al. (2007). An improved cell-free system for picornavirus synthesis. *J. Virol. Methods* 142(1-2):182-8.
3. Kozak, M. (2005). Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene* 361:13-37.
4. Kozak, M. (1983). Comparison of initiation of protein synthesis in prokaryotes, eukaryotes, and organelles. *Microbiol. Rev*. 47(1):1-45.
5. Mikami, S., et al. (2006). An efficient mammalian cell-free translation system supplemented with translation factors. *Protein Expr. Purif*. 46(2):348-57.

## 1-Step Human High-Yield In Vitro Translation Kits

Express up to 0.75mg per mL of functional protein in less than 16 hours.

The 1-Step Human High-Yield *In Vitro* Translation (IVT) Kits enable the expression of functional proteins with 10 to 100 times greater yield per mL than other mammalian IVT systems.

The 1-Step High-Yield IVT System uses modified HeLa cell extracts to take advantage of the robust human translation machinery and generate functional full-length proteins. In this system, protein expression is performed in a proprietary dialysis device that allows a continuous supply of nucleotides, amino acids and energy generating substrates into the reaction while removing inhibitors of proteins synthesis. This continuous-exchange cell-free (CECF) system enables protein expression in an overnight incubation of up to 750µg/mL. The complete mini- and maxi-scale kits include all the components required for transcription and translation of a recombinant gene, including an optimized expression vector.



### Highlights:



- **High expression** – up to 750µg/mL of expressed protein
- **Reproducibility** – low variability between experiments
- **Fast** – express high levels of protein with 6 hours to overnight incubation
- **Functional** – obtain functionally active proteins, including those containing disulfide bonds
- **Easy-to-use** – transcription and translation is performed in one reaction step
- **Adapt for labeling** – amenable to incorporation of [heavy](#) or unnatural amino acids

Includes: Complete kits contain cell lysate, reaction mix, accessory proteins, 5X dialysis buffer, control DNA, cloning vector, dialysis devices and centrifuge tubes

Requires: 30°C incubator or water bath (shaker incubator increases expression by 30 to 50%)

### Applications:

- Production of recombinant proteins for structural analysis
- Generate active proteins for protein interaction studies
- Perform rapid expression and characterization of mutant proteins
- Small-scale production of viral particles
- Incorporation of unnatural amino acids or toxic protein production

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	<a href="#">Price</a>
88890	<b>1-Step Human High-Yield</b>	2 x 100µL kit				
88891	<b>Mini IVT Kit</b>	10 x 100µL kit				
88892	<i>Sufficient For: 2 reactions of 100µL each</i>	2 x 2mL kit				
	Kit Contents (small kit): HeLa Lysate, 110µL Accessory Proteins, 25µL Reaction Mix, 40µL 5X Dialysis Buffer, 600µL Positive Control DNA (pCFE-GFP), 10µg pT7CFE1-NHis-GST-CHA, 10µg Microdialysis Device, 2 each Nuclease-free Water, 5mL					

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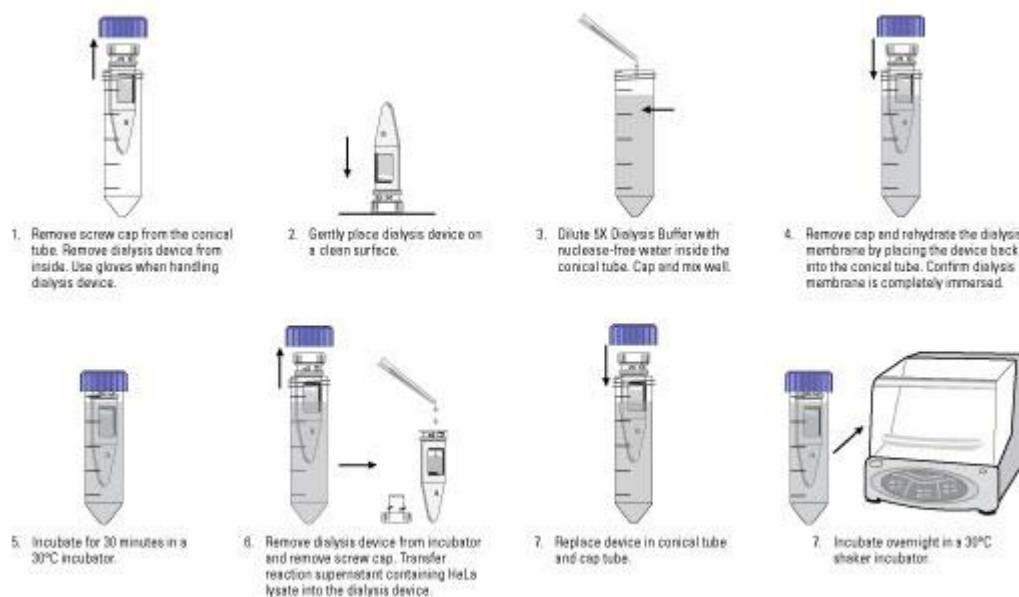
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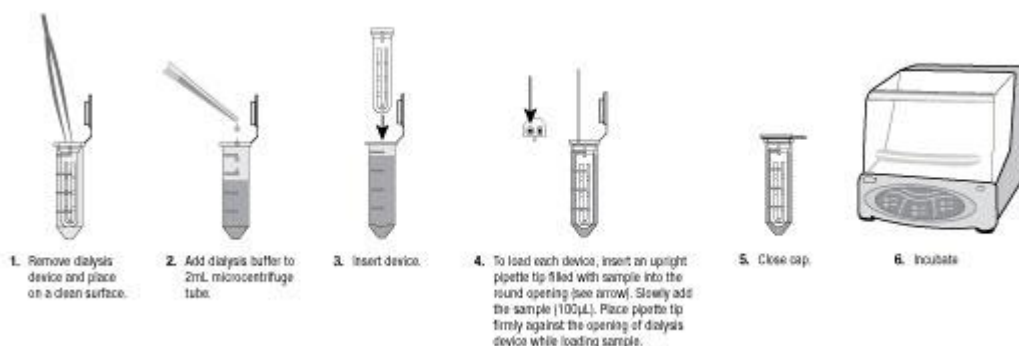
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## Product Details:

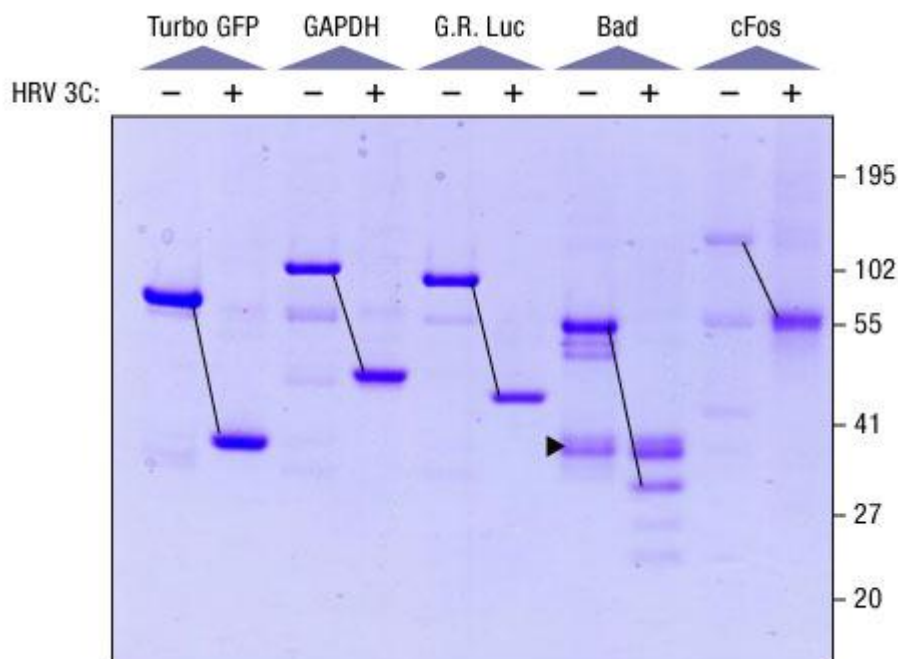


**Procedure summary for the Maxi IVT kit.** (Click image to view larger.) The reaction is performed in a dialysis device inserted into a 50mL conical tube. The first five steps are for equilibration and preparation of the dialysis device. The IVT reaction mixture is added in step 6.

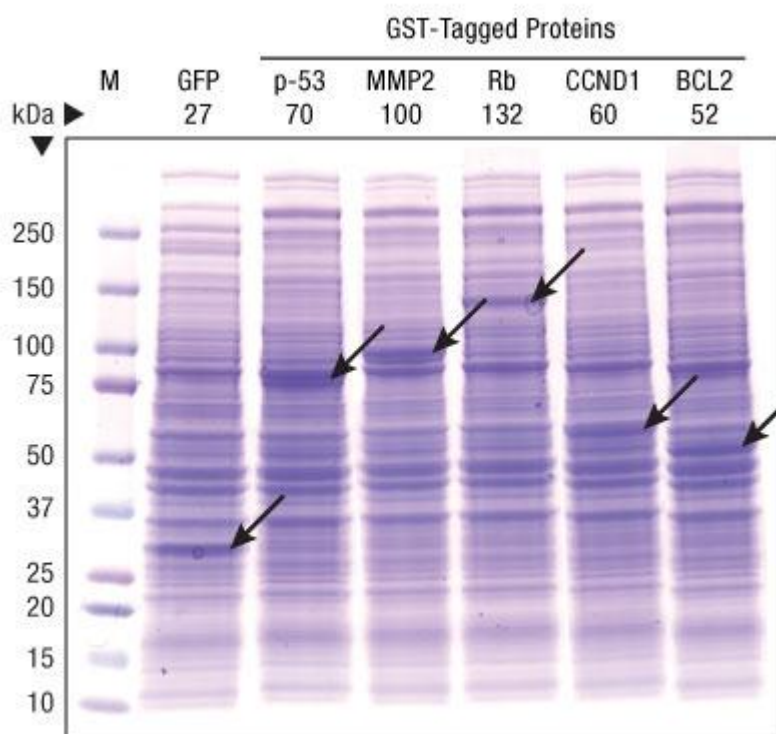


**Procedure summary for the Mini IVT kit.** (Click image to view larger.) The reaction is performed in a microdialysis device inserted into a 2mL microcentrifuge tube.

The 1-Step Human High-Yield IVT Kits are cell-free protein expression systems that provide all of the essential components required for transcription and translation. The kits are optimized with Accessory Proteins and Reaction Mixes that support protein synthesis using a DNA template. The advantages of using the 1-Step Human High-Yield IVT Kits over traditional *in vivo* expression systems include the ability to express toxic or insoluble proteins, easily perform protein labeling with modified amino-acids and reduce the time and cost of expressing human proteins in tissue culture cells. 1-Step Human IVT Kits are optimized for use with the pT7CFE1 expression vector that utilizes the T7 viral promoter and an EMCV Internal Ribosome Entry Site (IRES) to facilitate high levels of *in vitro* protein expression. Using a pT7CFE vector is critical for obtaining high expression levels. For added convenience, a family of pT7CFE vectors are available with tandem affinity tags for facilitate protein detection and purification. (For smaller scale and screening options, consider the 1-Step Human Coupled IVT Kit.)



**Purification of GST fusion proteins expressed by the 1-Step Human High-Yield IVT Kit.** Proteins were purified using glutathione agarose with elution by either 10mM glutathione or [HRV3C protease](#) cleavage, respectively. In the lanes for Bad protein, the additional bands (triangle) are 14-3-3 proteins, which co-elute with Bad. Protein identification was verified by mass spectrometry (data not shown). These purification strategies are described in greater detail in a [related article](#).



**Expression of coomassie-stainable levels of proteins using the 1-Step Human High-Yield IVT Kit.** Five expression-ready clones (pANT7-based vector) obtained from the DNASU Plasmid Repository were used to express the GST-fusion proteins listed in Lanes 3-7. Lane 2 shows expression of the control pCFE-GFP plasmid. Reaction mixtures of 5µL were separated by SDS-PAGE and stained with GelCode Blue Stain Reagent (Part No. 24590). Arrows indicate the positions of expressed proteins.

**Protein yields for each 1-Step Human High-Yield IVT Kit.** Expected yield is for the positive control GFP protein (vector included in each kit).

Kit	Size(s)	Reaction Volume	Expected Yield
1-Step Human High-Yield Mini IVT Kit	2-rxn and 10-rxn kits	0.1mL	0.25mg/mL
1-Step Human High-Yield Maxi IVT Kit	2-rxn kit	2mL	0.25mg/mL

#### Product References:

1. Heidary DK, Glazer EC. (2014). A light-activated metal complex targets both DNA and RNA in a fluorescent *in vitro* transcription and translation assay. *Chembiochem*. 15(4):507-11.
2. Festa F., et al. (2013) Robust microarray production of freshly expressed proteins in a human milieu. *Proteomics Clin Appl*. 7(5-6):372-7. **Published by Joshua LaBaer's group.**
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6. Stergachis, A. (2011). Rapid empirical discovery of optimal peptides for targeted proteomics. *Nature Methods*, 8:1041-43.
7. Wang Q. Y. et al. (2011) A translation inhibitor that suppresses dengue virus *in vitro* and *in vivo*. *Antimicrob Agents Chemother*. 55:4072-80.
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3. Kozak, M. (2005). Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene* 361:13-37.
4. Kozak, M. (1983). Comparison of initiation of protein synthesis in prokaryotes, eukaryotes, and organelles. *Microbiol. Rev*. 47(1):1-45.
5. Mikami, S., et al. (2006). An efficient mammalian cell-free translation system supplemented with translation factors. *Protein Expr. Purif*. 46(2):348-57.





## 1-Step Heavy Protein In Vitro Protein Expression Kits

*Express full-length heavy proteins for mass spectrometry proteomics analysis.*

1-Step Heavy Protein *In Vitro* Protein Expression Kits enable rapid cell-free expression of recombinant proteins containing stable isotope-labeled (i.e., heavy) amino acids.

The 1-Step Heavy Protein IVT Kit uses a unique HeLa cell lysate supplemented with heavy amino acids for *in vitro* translation (IVT) of proteins with 90 to 95% isotope incorporation efficiency in less than 8 hours. Heavy proteins expressed using this system can be used as mass spectrometry controls for sample prep loss, digestion efficiency determination or as quantification standards.



### Highlights:

- **Efficient** – express heavy proteins with 90 to 95% stable isotope incorporation
- **Functional** – uses the human translational machinery to express more biologically active proteins than other IVT systems
- **Flexible** – express light proteins or heavy proteins containing  $^{13}\text{C}_6\ ^{15}\text{N}_2$  L-lysine and/or  $^{13}\text{C}_6\ ^{15}\text{N}_4$  L-arginine
- **Convenient** – perform transcription and translation in a single step
- **High performance** – greater yields compared to rabbit reticulocyte *in vitro* translation

### Applications:

- Identify ideal peptides for targeted quantitation
- Verify protein digestion efficiency
- Control for protein sample prep variability and affinity enrichment loss

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	<a href="#">Price</a>
88330	1-Step Heavy Protein IVT Kit	8-rxn kit				
88331	Formulation: Multi-component kit <i>Sufficient For: 8 reactions of 25μL each</i>  Kit Contents (8-rxn kit): HeLa Lysate, 110μL Accessory Proteins, 25μL Reaction Mix, 40μL 13C6 15N4 L-Arginine, 25μL 13C6 15N2 L-Lysine, 25μL Positive Control DNA: pCFE-GFP, 10μg pT7CFE1-CGST-HA-His, 10μg Nuclease-Free Water 1.5mL	40-rxn kit				



## Product Details:

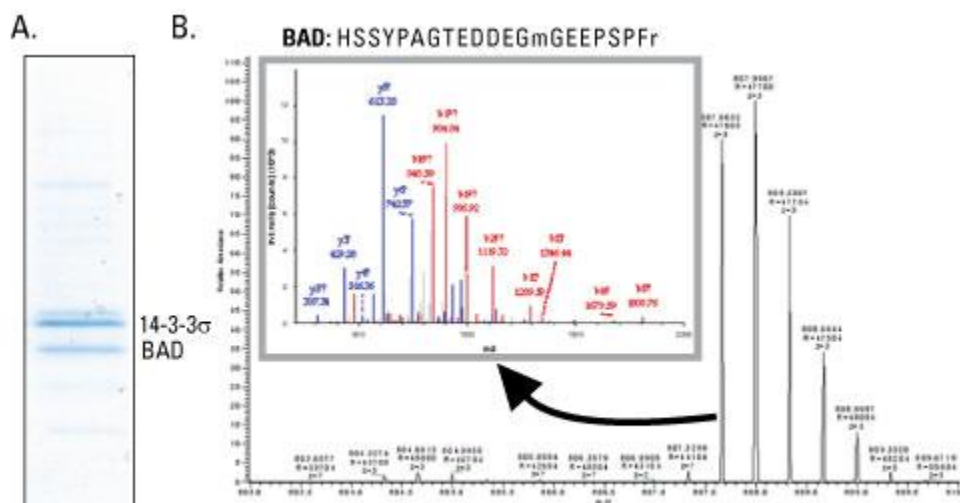
1-Step Heavy Protein IVT Kit contains all of the necessary components to express a heavy protein including HeLa cell lysate, proprietary accessory proteins, reaction mix, heavy amino acids, positive-control GFP DNA and the pT7CFE1-CGST-HA-His cloning vector. The benefits of *in vitro* expression of heavy proteins over traditional *in vivo* systems include expression of toxic or insoluble proteins, a more rapid protein synthesis and a more economical use of heavy amino acids compared to stable-isotope labeled cell lines. This small-scale expression method makes it easy to express numerous heavy proteins simultaneously or to express large quantities of a single heavy protein up to 100µg/mL. Tandem affinity purification of heavy proteins is aided using an expression vector containing multiple affinity tags including GST, HA and 6xHis.

### Expression and analysis of stable-isotope labeled BAD protein with the 1-Step Heavy Protein IVT Kit. A.

HA-tagged BAD was expressed using the 1-Step Heavy Protein IVT Kit and affinity purified using the HA Tag IP/Co-IP Kit and analyzed by SDS-PAGE. B.

The protein bands were excised, digested and analyzed using a LTQ Orbitrap XL mass spectrometer and identified as heavy BAD and light 14-3-3σ which co-purified during immunoprecipitation. MS

spectrum of a light and heavy BAD peptide showing >95% heavy isotope incorporation. MS/MS spectrum used for peptide identification is shown in the figure insert.



**Efficient heavy amino acid incorporation.** Percent heavy amino acid incorporation into recombinant proteins expressed using the 1-Step Heavy Protein IVT Kit.

Expressed Protein	Incorporation
BAD	92%
CCND1	97%
TP53	91%
RB	96%
GAPDH	94%
GFP	95%

## References:

1. Mikami, S., et al. (2006). An efficient mammalian cell-free translation system supplemented with translation factors. *Protein Expr. Purif.* 46(2):348-57.
2. Mikami, S., et al. (2008). A human cell-derived in vitro coupled transcription/translation system optimized for production of recombinant proteins. *Protein Expr. Purif.* 62(2):190-8.
3. Hanke S, et al. (2008). Absolute SILAC for accurate quantitation of proteins in complex mixtures down to the attomole level. *J. Proteome Res.* 7(3):1118-30.
4. Ciccimaro E., et al. (2009). Absolute quantification of phosphorylation on the kinase activation loop of cellular focal adhesion kinase by stable isotope dilution liquid chromatography/mass spectrometry. *Anal. Chem.* 81(9):3304-13.
5. Stergachis, A., et al. (2011) Rapid empirical discovery of optimal peptides for targeted proteomics. *Nat. Meth.* 8(12):1041-1043.

## T7 Cell-Free Expression Vectors (pT7CFE1)

*Optimized for in vitro transcription and translation of fusion proteins.*

T7 Cell-Free Expression Vectors (pT7CFE1) are cloning plasmids optimized to use with the 1-Step Human In Vitro Protein Expression System for in vitro translation (IVT) of tagged fusion proteins.

The pT7CFE1 Expression Vectors contain the Encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) element that is critical for high levels of cap-independent protein expression in the Human In Vitro Translation System. Each vector features a highly-compatible multiple cloning site (MCS) to facilitate easy insertion of protein coding sequences into and between vectors. The pT7CFE1 Vector is available with single or tandem affinity tags at the N- or C- terminus to facilitate protein purification and detection. The pT7CFE Vectors are suitable for insertion of cloned genes, cDNAs, ORFs or PCR products for in vitro transcription and translation. Custom cloning services are also available.











### Highlights:

- **Optimized performance** – designed to provide the highest yield in the human *in vitro* translation system
- **Many options** – multiple tag and tag-location options available
- **Modular MCS** – multiple cloning site is maintained across vector family to facilitate subcloning
- **Cleavable tags** – HRV 3C cleavage site available on select vectors

### pT7CFE1 Plasmid Features:

- EMCV IRES at the 5' UTR promotes high-level translation of mRNAs
- MCS accommodates gene insertion via ten different restriction sites: MscI, NdeI, BamHI, EcoRI, EcoRV, PacI, PstI, SacI, SalI, NotI and XhoI
- Poly A sequence in the 3' region promotes mRNA stabilization and protection from nucleases
- T7 terminator ensures synthesis of accurate size mRNA transcripts
- Plasmid linearization may be accomplished with restriction sites between Poly A sequence and the T7 terminator region

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	<a href="#">Price</a>
88859	pT7CFE1-NHis Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg				
88860	pT7CFE1-CHis Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg				
88861	pT7CFE1-NHA Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg				
88862	pT7CFE1-CHA Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg				

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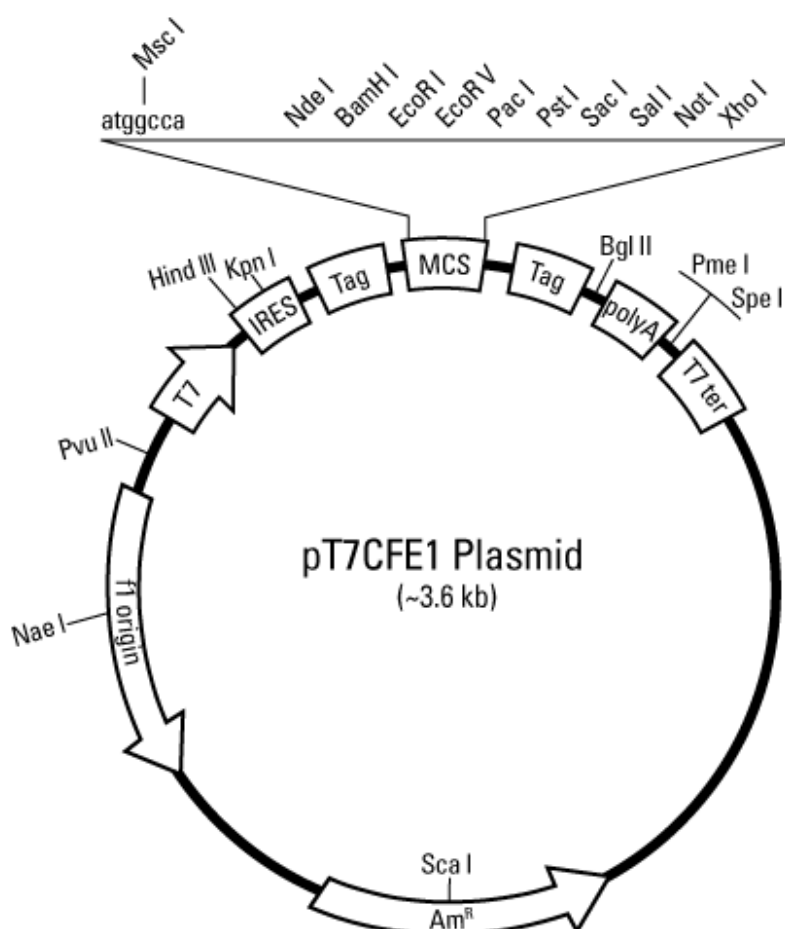
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88863	pT7CFE1-NMyc Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88864	pT7CFE1-CMyc Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88865	pT7CFE1-NFtag Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88866	pT7CFE1-CFtag Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88867	pT7CFE1-NHA-CHis Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88868	pT7CFE1-CGST-HA-His Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88869	pT7CFE1-CGFP-HA-His Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88870	pT7CFE1-NHis-GST Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		
88871	pT7CFE1-NHis-GST-CHA Formulation: 0.5µg/µL DNA in 10mM Tris-HCl, pH 8.5	10µg		

### Product Details:

Map of the Thermo Scientific T7 Cell-free Expression Vector elements. Data sheets with features and maps of individual plasmids and complete sequence information are available for download (see table).



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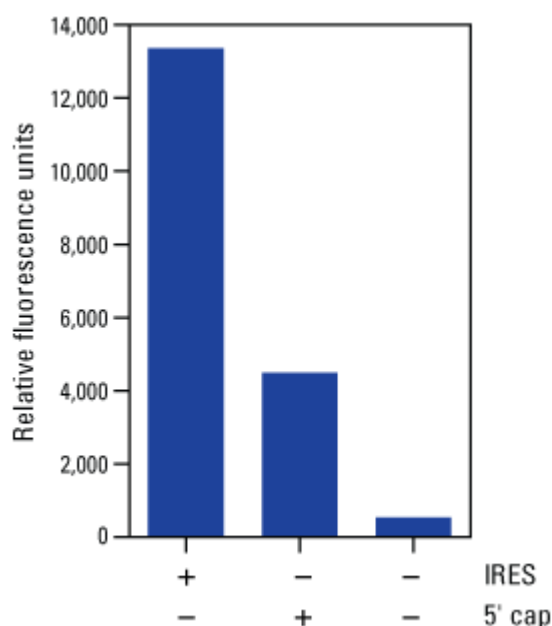
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**pT7CFE1 Vectors for cell-free protein expression and available affinity and cleavage tag options.**

Vector	N-term Tag	C-term Tag	Cleavage Site	Vector Information Files	
pT7CFE1-NHis	6xHis			<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-CHis		6xHis		<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NHA	HA			<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-CHA		HA		<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NMyc	c-Myc			<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-CMyc		c-Myc		<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NFtag	Ftag			<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-CFtag		Ftag		<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NHA-CHis	HA	6xHis		<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-CGST-HA-His		GST HA 6xHis	HRV 3C (C-term)	<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFF1-CGFP-HA-His		GFP HA 6xHis	HRV 3C (C-term)	<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NHis-GST	9xHis GST		HRV 3C (N-term)	<a href="#">Data sheet</a>	<a href="#">Sequence</a>
pT7CFE1-NHis-GST-CHA	9xHis GST	HA	HRV 3C (N-term)	<a href="#">Data sheet</a>	<a href="#">Sequence</a>



**IRES-mediated protein expression is significantly greater than 5' capped mRNA.** tGFP mRNA was transcribed from the pCFE expression plasmids containing an upstream IRES element (+ IRES) or another plasmid without an IRES element. The mRNA generated without an IRES element was either used directly (- IRES, - cap) or chemically modified to have an N-terminal Anti-Reverse Cap Analog (ARCA, + 5' cap). Equal amounts of all the three mRNA's were used in human *in vitro* translation reactions for 2 hours at 30°C and the relative amount of GFP was determined by fluorescence.

**Product References:**

Yadavalli, R., Ledger, C., Sam-Yellowe, TY. (2012). In vitro human cell-free expression system for synthesis of malaria proteins. Parasitol Res. 2012 Jul 11. [Epub ahead of print] DOI 10.1007/s00436-012-3014-7.



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