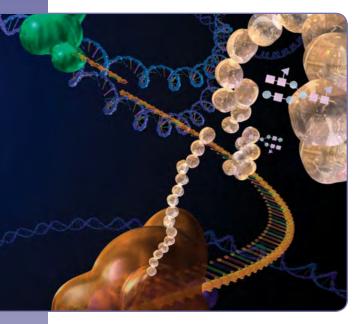


## 1 step to functional proteins

1-Step Human Coupled *In Vitro* Translation System • 1-Step Human High-Yield *In Vitro* Translation System • 1-Step Heavy Protein *In Vitro* Translation System



# Why is a human IVT system better?



#### **Easy protocol**

The Thermo Scientific 1-Step Human *In Vitro* Translation (IVT) System synthesizes functional proteins using the human translation machine. The 1-Step Human IVT System couples DNA transcription and translation, delivering protein in as little as 90 minutes. Optimal protein expression is achieved using our pT7CFE1 family of vectors, which contain the proper transcription and translation elements. This system uses a T7 RNA Polymerase to drive efficient transcription, coupled with HeLa cell-free extracts to drive translation.

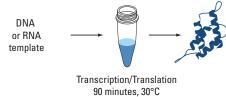


Figure 1. *In vitro* translation with Thermo Scientific 1-Step Human IVT System.

Compared to traditional methods for protein expression, human in vitro translation enables researchers to:

- Express protein in minutes without the need to grow and transfect cells
- Generate protein with post-translational modifications
- Easily express a large number of clones
- Label proteins efficiently with unnatural amino acids
- Generate proteins which are cytotoxic or difficult to express in a cell-based system

The 1-Step Human In Vitro Translation System Selection Guide.

	1-Step Human Coupled <i>In Vitro</i> Translation System	1-Step Human High Yield <i>In Vitro</i> Translation System	1-Step Heavy Protein <i>In Vitro</i> Translation System	
1-Step reaction volume	25μL	0.1, 0.25 or 1mL	25μL	
Protein yield (concentration)	40-100μg/mL	250-750µg/mL	40-100μg/mL	
Reaction time	90 min - 6 hrs	6-24 hrs	90 min - 6 hrs	
Applications	Mutational analysis     Electrophoretic Mobility Shift     Assays (EMSA)     Co-Immunoprecipitation (Co-IP)     High-throughput protein     expression     Express cytotoxic proteins     Unnatural amino acid     incorporation     Screening for translation     inhibitors     Identifying optimal peptide     fragments for analysis by     mass spec³	Overnight expression of milligram quantity of protein     Express proteins difficult to generate in traditional systems due to cytotoxicity or protein denaturation     Incorporate unnatural amino acids     Structural analysis	Incorporation of isotopic amino acids     Controls in mass spectrometry analysis for sample prep loss or protein digestion     Generating quantification standards	

#### Higher protein activity

Functional activity is based on protein structure and post-translational modifications. Comparison of the expression of the green *Renilla* luciferase enzyme in *E. coli* cultures and in the 1-Step Human High Yield *In Vitro* Translation System demonstrates 25 times greater luciferase activity in the human *in vitro* translation system, due to improved protein folding (Figure 2).

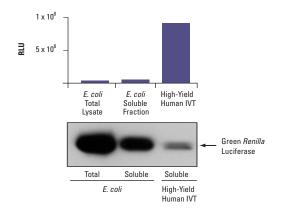


Figure 2. Higher levels of green *Renilla* luciferase activity when expressed in the Thermo Scientific 1-Step Human IVT System. Green *Renilla* luciferase expressed in *E. coli* cells-lysate samples was then either used directly (Total) or clarified at  $10,000 \times g$  for 5 minutes (Soluble). *In vitro* translation of green *Renilla* luciferase was done using the 1-Step Human High Yield IVT Kit (Product # 88886). Activity was measured using the Thermo Scientific Pierce Green *Renilla* Luciferase Flash Assay Kit (Product # 16164) and Western blots were carried out using  $2\mu L$  of each of indicated samples. RLU = relative luciferase units.

#### Higher protein yield

Compared to rabbit reticulocyte-based systems, any of our HeLa-based *in vitro* translation systems delivers higher protein yields with sustained expression for longer periods of time. Additionally, rabbit reticulocyte-based systems can produce background signals in Western blots because of cross reactivity with common Western blot antibodies, such as the globulin signal (Figure 3).

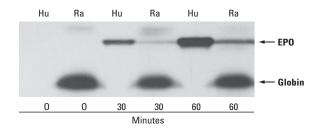


Figure 3. Increased Erythropoietin (EPO) expression using the Thermo Scientific 1-Step Human IVT Kit compared to rabbit reticulocyte-based systems. HA-tagged EPO expression was carried out using 1-Step Human Coupled IVT Kit (Hu) and the rabbit reticulocyte (Ra)-based system according to the manufacturers' instructions. 2µL of samples were removed from each reaction at indicated times and analyzed for EPO expression in a western blot using anti-HA antibodies.

#### Easier assay development

DUESESS

The absence of heme protein in our human translation reaction eliminates interference with assays using fluorescent or colorimetric signal. Translation reactions containing green fluorescent protein (GFP) mRNA were performed with the 1-Step Human IVT System (Human) and a leading rabbit reticulocyte lysate system (Rabbit), followed by fluorescent detection. (Figure 4).

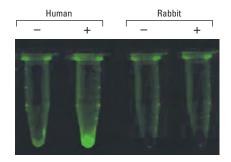


Figure 4. *In vitro* protein expression in a human system enables easy detection of fluorescent proteins. Translation reactions containing green fluorescent protein (GFP) mRNA were performed with the Thermo Scientific 1-Step Human *In Vitro* System (Human) and a leading rabbit reticulocyte lysate system (Rabbit). Expression of tGFP was easily monitored directly in reaction tubes using a FITC filter. The 1-Step Human IVT System is compatible with fluorescence and colorimetric protein detection. The rabbit reticulocyte lysate system interferes with fluorometric as well as colorimetric detection.

#### **Faster protein expression**

The time required to express a protein in tissue culture cells is longer than *in vitro* methods. Additionally, cytotoxic proteins fail in tissue culture methods compared to *in vitro* translation (Figure 5).

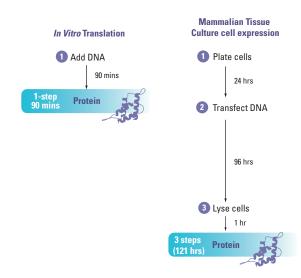


Figure 5. Faster protein expression with human *in vitro* translation system versus expression in mammalian cell culture.

### 1 step to functional protein



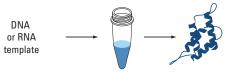
### Thermo Scientific 1-Step Human Coupled *In Vitro* Translation System

The 1-Step Human Coupled IVT Kit is a HeLa cell lysate-based protein expression system ideal for generating microgram quantities of protein. Protein expression is performed in a single 90-minute reaction that can be extended for up to six hours, with continued protein production up to 100µg/mL when combined with the optimized pT7CFE1 Expression Vector (included with each kit). Expression can be driven directly from our pT7CFE expression vector (DNA kits) or from mRNA transcripts (RNA kits).

The 1-Step Human IVT System is ideal for expressing functional enzymes, phosphoproteins and glycoproteins; studying protein interactions; and performing rapid mutational analysis. Certain membrane proteins with 1-3 transmembrane domains have also been produced. This format is amendable to high throughput protein expression and has been used to express protein libraries for the identification of peptide fragments optimized for mass spectrometry detection<sup>3</sup>.

#### **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



Transcription/Translation 90 minutes, 30°C

Figure 6. *In vitro* translation with Thermo Scientific 1-Step Human IVT System.

#### **Protein yield**

Luciferase was expressed using the 1-Step Human Coupled *In Vitro* Translation System and compared to yields in rabbit reticulocyte-based systems. Protein yields from the 1-Step Human IVT Kit were much higher as measured by luciferase assay and Western blotting (Figure 7).

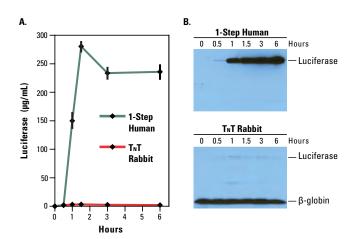


Figure 7. The Thermo Scientific 1-Step Human Coupled IVT Kit produces more active protein without interfering substances. *In vitro* luciferase expression reactions were performed with the 1-Step Human Coupled IVT Kit and the rabbit reticulocyte-based TnT 77 Quick Coupled Transcription/Translation System according to supplied instructions. 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-clobin.

#### **Protein labeling**

Luciferase was expressed using the 1-Step Human Coupled *In Vitro* Translation System, and either fluorescent (Figure 8A and B) or radioactive (Figure 8C) amino acids were incorporated. Compared to proteins expressed in a rabbit reticulocyte system, detection of either fluorescent or radioactive signal was enhanced in protein expressed in the human IVT system resulting from higher protein yields.

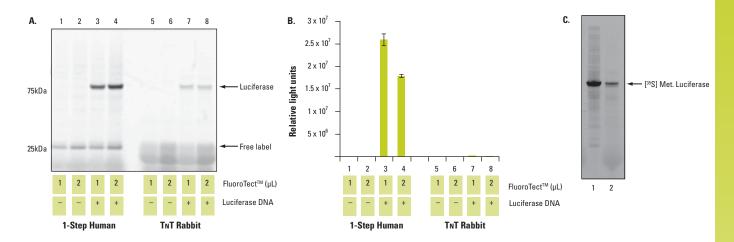


Figure 8. Improved labeling and activity of luciferase expressed in the Thermo Scientific 1-Step Human Coupled IVT Kit compared to rabbit reticulocyte-based  $T_NT$  system. Luciferase was expressed in the 1-Step Human Coupled IVT Kit (Product # 88882) with the following modifications:  $25\mu L$  reaction containing 50% human cell lysate,  $2.5\mu L$  accessory proteins,  $5\mu L$  reaction mix,  $1\mu g$  firefly luciferase in pCFE1-CHis Vector, 1 or  $2\mu L$  of Fluorotect  $^{\text{tot}}$  BODIPY®-conjugated lysine for 3 hours. Reactions containing rabbit reticulocyte lysates were exactly carried out according to the manufacturing instructions except that incubations were carried out for 3 hours. **A:**  $2\mu L$  of samples were run on a 4-12 % SDS-PAGE gel and analyzed for

BODIPY labeled luciferase using a Typhoon® instrument with a 532 excitation. **B:** 2µL of each translation reaction was analyzed for luciferase activity using the Thermo Scientific Firefly Luciferase Assay Kit (Product # 16174). **C:** Comparison of incorporation of [\*S]-methionine into luciferase. Radioactive labeling of luciferase was performed with the 1-Step Human Coupled IVT Kit (Lane 1) and the rabbit reticulocyte-based T<sub>N</sub>T T7 Quick Coupled Transcription/Translation System (Lane 2) according to manufacturers instructions and control plasmids using 1µL of [\*S]-L-methionine. Samples were removed from each reaction at the 90 min. separated by SDS-PAGE gels, dried and exposed to X-ray film.

#### **Ordering Information**

All kits below contain:
HeLa Lysate
Accessory Proteins
Reaction Mix
Positive Control DNA: pCFE-GFP
pT7CFE1-CHis
Nuclease-free Water

Product #	Description	Pkg. Size
88881	1-Step Human Coupled IVT Kit - DNA Sufficient for 8 reactions of 25µL each.	8-rxn kit
88882	1-Step Human Coupled IVT Kit - DNA Sufficient for 40 reactions of 25µL each.	40-rxn kit
88883	1-Step Human IVT Kit - mRNA Sufficient for 8 reactions of 25µL each.	8-rxn kit
88884	1-Step Human IVT Kit - mRNA Sufficient for 40 reactions of 25µL each.	40-rxn kit

### 1 step to higher yields



### Thermo Scientific 1-Step Human High-Yield *In Vitro* Translation System

The 1-Step High-Yield IVT System uses modified HeLa cell extracts to take advantage of the robust human transcription and translation machinery and generate functional full-length proteins. In this system, protein expression is performed in a unique 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 system enables protein expression for up to 24 hours and produces protein yields ranging from 250 to 750µg/mL. The complete kits come with all components required for proper transcription and translation of recombinant protein, including a pT7CFE1 Expression Vector. Proteins made in the 1-Step High-Yield System can be purified using tag-specific resins and the tag can be cleaved by using HRV3C.

#### **Highlights:**

- **High expression** up to 750µg/mL of expressed protein
- Reproducibility low variability between experiments
- Fast express high levels of protein with an overnight incubation
- Functional obtain more active protein than with bacterial, rabbit or wheat germ protein expression

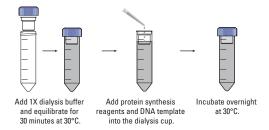


Figure 9. Procedure for Thermo Scientific 1-Step Human High-Yield In Vitro Translation.

#### **Protein yield**

The 1-Step Human High-Yield IVT System is capable of expressing much higher levels of protein than traditional mammalian *in vitro* expression systems (Figure 10).

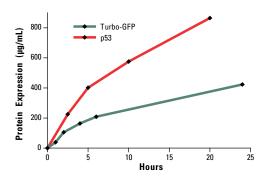


Figure 10. In vitro expression of TurboGFP™ and p53 using the Thermo Scientific 1-Step Human High-Yield IVT Kit. Recombinant p53 and GFP proteins were expressed using the 1-Step Human High-Yield Mini-IVT Kit. At each time point, 5µL aliquots were withdrawn from the reactions and stored at -20°C until assayed. The amount of p53 was quantified by ELISA and TurboGFP was quantified using a fluorescent plate-based assay.

#### **Enzyme activity**

*Cypridina* luciferase was expressed in the 1-Step Human High-Yield IVT System, and compared to *E. coli* expression (Figure 11). Luciferase activity was 1,000 times higher in the human-expressed *Cypridina* because of the ability of the human system to fold the enzyme properly and form the 17 disulfide bridges.

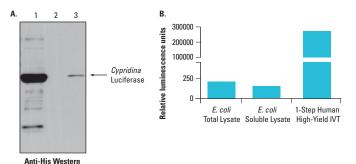
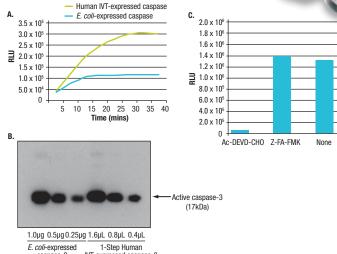


Figure 11. *Cypridina* luciferase is produced with higher functional activity in the Human 1-Step High-Yield IVT Kit compared to *E. coli* expression cultures. A: His-tagged *Cypridina* luciferase was expressed in *E. coli* cultures and in the 1-Step Human High-Yield IVT system. Equal volumes of either *E. coli* cell lysate or human IVT reaction expressing *Cypridina* luciferase were immunoblotted for luciferase. Lane 1, *E. coli* total lysate; Lane 2, *E. coli* soluble fraction (clarified a 10,000 x *g* for 5 min); Lane 3, 1-Step Human High-Yield IVT. B: Activity of *Cypridina* luciferase was assessed in equal volumes of either *E. coli* total lysate, *E. coli* soluble fraction or the 1-Step Human High-Yield IVT reactions using the Thermo Scientific Pierce *Cypridina* Luciferase Assay Kit (Product #16168). These results indicate that *Cypridina* luciferase expressed in the 1-Step Human High-Yield IVT System has significantly greater activity than that expressed in *E. coli* cultures.



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Figure 12. Higher activity of human caspase-3 expressed in Thermo Scientific 1-Step Human High-Yield IVT System versus *E. coli*. Panel A. Equal amounts of human caspase-3 either expressed in *E. coli* or using 1-Step Human High-Yield IVT Kits were assayed for active caspase-3 activity using caspase-3 glo® assay reagent mix containing cleavable DEVD-aminoluciferin and luciferase substrate. *E. coli*-expressed caspase-3 is supplied at 5.88 units/mg of protein. Panel B. Caspase-3 expressed in either *E. coli* or human IVT system tends to undergo self-proteolysis resulting in 17kDa and 12kDa proteins. Indicated amounts of recombinant caspase-3 proteins or volumes from the total reaction expressing caspase-3 from 1-Step Human High-Yield IVT Kits were separated on SDS-PAGE and Western blotting carried out using active-caspase-3 antibodies and Thermo Scientific SuperSignal Chemiluminescent Pico Substrate. Panel C. 0.1μL of 1-Step Human High-Yield IVT-expressed caspase-3 was incubated with 1μM of either a caspase-3 specific inhibitor Ac-DEVD-CHO or a negative control A-FA-FMK inhibitor for 10 minutes and measured for caspase-3 activity using caspase-3 glo assay reagent. None indicates no chemical added before measuring for luciferase activity.

#### **Ordering Information**

All kits below contain:
HeLa Lysate
Accessory Proteins
Reaction Mix
5X Dialysis Buffer
Positive Control DNA: pCFE-GFP
pT7CFE1-CGST-HA-His
Slide-A-Lyzer® MINI Dialysis Device
Cap Lock
Pierce® Microcentrifuge Tube
Nuclease-free Water

Product #	Description	Pkg. Size
88885	1-Step Human High-Yield Mini IVT Kit Sufficient for 2 high-yield reactions of 100µL each (approx. 50µg of GFP protein total.)	2-rxn kit
88886	1-Step Human High-Yield Mini IVT Kit Sufficient for 10 high-yield reactions of 100µL each (approx. 250µg of GFP protein total.)	10-rxn kit
88887	1-Step Human High-Yield Midi IVT Kit Sufficient for 8 high-yield reactions of 250µL each (approx. 500µg of GFP protein total.)	8-rxn kit
88889	1-Step Human High-Yield Maxi IVT Kit Sufficient for 4 high-yield reactions of 1mL each (approx. 1mg of GFP protein total.)	4-rxn kit

#### **Related Products**

Product #	Description	Pkg. Size
88221	HisPur Ni-NTA Resin Sufficient for binding up to 60mg of His-tagged protein per mL of resin.	10mL
89964	HisPur Cobalt Resin Sufficient for binding >10mg of His-tagged protein per mL of resin.	10mL
16100	Pierce Glutathione Agarose Sufficient for binding > 10mg of GST-tagged protein per mL of resin.	10mL
26182	Pierce Anti-HA Agarose Sufficient for binding >60nmol HA-tagged protein per mL of resin.	10mL

Please visit thermoscientific.com/pierce to discover more package sizes and formats of these resins.

### 1 step to heavy proteins



### Thermo Scientific 1-Step Heavy Protein *In Vitro* Translation System

Thermo Scientific 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 of proteins with 90 to 95% isotope incorporation efficiency in less than eight 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 90-95% 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 <sup>13</sup>C<sub>6</sub><sup>15</sup>N<sub>2</sub> L-lysine and/or <sup>13</sup>C<sub>6</sub><sup>15</sup>N<sub>4</sub> L-arginine
- Convenient perform transcription and translation in a single step
- **High performance** greater yields compared to rabbit reticulocyte *in vitro* translation

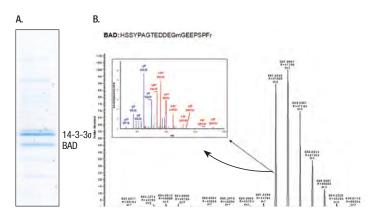


Figure 13. Expression and analysis of stable-isotope labeled BAD protein with the Thermo Scientific 1-Step Heavy Protein IVT Kit. A: HA-tagged BAD was expressed using the 1-Step Heavy Protein IVT Kit and affinity purified using the Thermo Scientific Pierce HA Tag IP/Co-IP Kit and analyzed by SDS-PAGE. B: The protein bands were excised, digested and analyzed using a Thermo Scientific LTQ Orbitrap XL mass spectrometer and identified as heavy BAD and light 14-3-30 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.



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

#### **Ordering Information**

eletter oo

Product # Description		Pkg. Size	
88330	1-Step Heavy Protein IVT Kit Sufficient for 8 reactions of 25µL each. Kit Contents: HeLa Lysate, 110µL Accessory Proteins, 25µL Reaction Mix, 40µL  13C <sub>0</sub> -15N <sub>4</sub> L-Arginine, 25µL 25C <sub>0</sub> -15N <sub>2</sub> L-Lysine, 25µL Positive Control DNA: pCFE-GFP, 10µg pT7CFE1-CGST-HA-His, 10µg Nuclease-free Water 1.5mL	8-rxn kit	
88331	1-Step Heavy Protein IVT Kit Sufficient for 40 reactions of 25µL each. Kit Contents: HeLa Lysate, 550µL Accessory Proteins, 125µL Reaction Mix, 200µL  13 C <sub>0</sub> 15 N <sub>4</sub> L-Arginine, 25µL  25 C <sub>0</sub> 15 N <sub>2</sub> L-Lysine, 25µL Positive Control DNA: pCFE-GFP, 10µg pT7CFE1-CGST-HA-His, 10µg Nuclease-free Water 1.5mL	40-rxn kit	

## convenient vectors complete the family



#### Human In Vitro Translation Expression Vectors

Thermo Scientific T7 Cell-Free Expression Vectors (pT7CFE1) are expression plasmids optimized to use with the Thermo Scientific 1-Step Human *In Vitro* Protein Expression System. The pT7CFE1 Expression Vectors contain the Encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) element that is critical for high levels protein expression in the Human *In Vitro* Translation System. Each vector features an identical 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- and/or C-terminus to facilitate protein purification and detection. The pT7CFE Vectors are suitable for insertion of cloned genes, cDNAs, ORFs or PCR products. 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

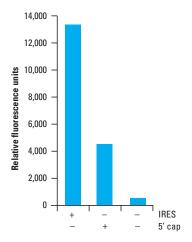


Figure 16. IRES-mediated protein expression is significantly greater than 5' capped mRNA. tGFP mRNA was transcribed from the pCFE expression plasmids containing an upstream IRES element (+ IRES) or another plasmid without an IRES element. The mRNA generated without an IRES element was either used directly (- IRES, - 5' 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.

#### Thermo Scientific T7 Cell-Free Expression Vectors Selection Guide.

For each order, 10 micrograms of vector is supplied at 0.5µg/µL in 10mM Tris-HCl, pH 8.5. Custom cloning services are also available.

Product #	Vector name	N-terminus Tag	C-Terminus tag	Cleavage site	
88859	pT7CFE1-NHis	6xHis	-		
88860	pT7CFE1-CHis	-	6xHis		
88861	pT7CFE1-NHA		-		
88862	pT7CFE1-CHA	-			
88863	pT7CFE1-NMyc	c-Myc	-		
88864	pT7CFE1-CMyc	-	c-Myc		
88865	pT7CFE1-NFtag	Flag <sup>™</sup> -tag	-		
88866	pT7CFE1-CFtag	-	Flag-tag		
88867	pT7CFE1-NHA-CHis	HA	6xHis		
88868	pT7CFE1-CGST- HA-His	-	GST, HA, 6xHis	HRV 3C	
88869	pT7CFF1-CGFP- HA-His	-	GFP, HA, 6xHis	HRV 3C	





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