INSTRUCTIONS

# 1-Step Human High-Yield Mini IVT Kit

88890 88891 2568.0

## Number Description

**88890** **1-Step Human High-Yield Mini IVT Kit,** contains sufficient reagents to perform 2 reactions (100µL each)

**88891** **1-Step Human High-Yield Mini IVT Kit,** contains sufficient reagents to perform 10 reactions (100µL each)

| Kit Contents | Cap Color | 88890X | 88891X |
|---|---|---|---|
| HeLa Lysate | Red | 110µL | 500µL |
| Accessory Proteins | Green | 25µL | 100µL |
| Reaction Mix | Yellow | 40µL | 200µL |
| 5X Dialysis Buffer | Blue/Clear | 600µL | 3mL |
| Positive Control DNA: pCFE-GFP (0.5µg/µL, 10µg) | Solid white | 20µL | 20µL |
| pT7CFE1-NHis-GST-CHA (0.5µg/µL, 10µg) | Clear | 20µL | 20µL |

| Kit Contents | 88890Y | 88891Y |
|---|---|---|
| Micro-dialysis Device | 2 each | 10 each |
| Nuclease-free Water | 5mL | 3 × 5mL |

**Note: Completely read the instructions before proceeding with the protocols.**

**Storage:** Upon receipt store Product 88890X or 88891X at -80°C and Product 88890Y or 88891Y at room temperature. Products 88890X and 88891X are shipped with dry ice. Products 88890Y and 88891Y are shipped at ambient temperature.

## Table of Contents


Introduction ..... 2
Procedure Summary ..... 2
Important Product Information ..... 2
Additional Materials Required ..... 2
Protocol for Using the 1-Step Human High-Yield Mini IVT Kit ..... 3
A. Protein Expression ..... 3
B. Determination of Protein Expression Level ..... 4
C. Purification of IVT-expressed Proteins ..... 4
Troubleshooting ..... 4
Additional Information ..... 5
A. pT7CFE1-NHis-GST-CHA Vector Cloning Sites and Sequence Features ..... 5
B. Vector DNA Clean-up and Concentration Protocol ..... 6
C. Expression-ready Clones for Use with the 1-Step Human High-Yield IVT Kit ..... 6
Related Thermo Scientific Products ..... 7
General References ..... 8



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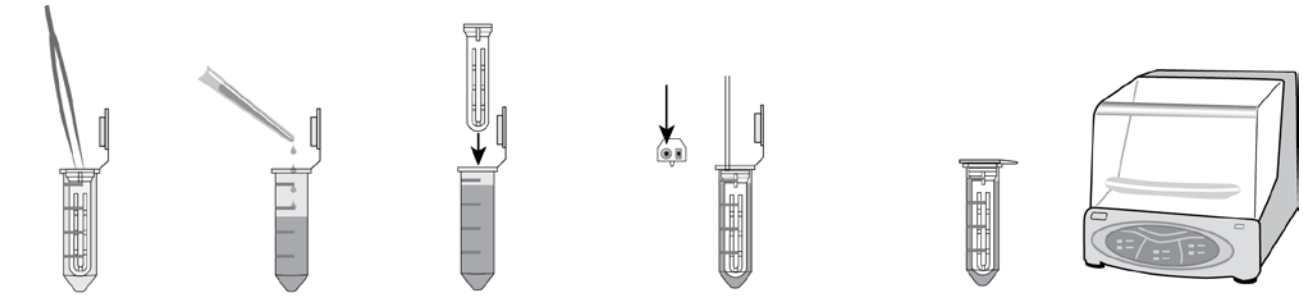
## Introduction

The Thermo Scientific™ 1-Step Human High-Yield Mini IVT Kit is a mammalian *in vitro* translation (IVT) system based on HeLa cell lysates, which contain all of the cellular components required for protein synthesis, including ribosomes, initiation factors, elongation factors and tRNA. When supplemented with the included proprietary Accessory Proteins, Reaction Mix and a DNA template cloned into the Thermo Scientific™ pT7CFE1-NHis-GST-CHA Vector, this system can synthesize protein for up to 16 hours. The benefits of *in vitro* protein expression over traditional *in vivo* systems include the ability to express toxic proteins, faster protein synthesis and protein labeling with modified amino acids. The optimized kit contains a T7 promoter and an EMCV Internal Ribosome Entry Site (IRES) to facilitate high levels of *in vitro* protein expression in a cap-independent fashion. Using a vector containing the EMCV IRES element is critical for obtaining high expression levels in this human *in vitro* protein expression system.

## Procedure Summary



1. Remove dialysis device and place on a clean surface.
2. Add dialysis buffer to 2mL microcentrifuge tube.
3. Insert device.
4. To load each device, insert an upright pipette tip filled with sample into the round opening (see arrow). Slowly add the sample (100µL). Place pipette tip firmly against the opening of dialysis device while loading sample.
5. Close cap.
6. Incubate

## Important Product Information

- **Use the included Thermo Scientific pT7CFE1-NHis-GST-CHA Vector (Product No. 88871) for cloning and expressing the target gene. See the Additional Information Section for additional vector choices, cloning sites and expression-ready clones.**
- Thaw HeLa Lysate on ice, aliquot and quickly store at -80°C. All of the kit components are stable for up to five freeze-thaw cycles as long as the contents are stored at -80°C immediately after use. For faster thawing, gently flick the lysate tubes.
- Undiluted lysate and reactions containing lysate will appear cloudy before and after incubation. Accessory Proteins and Reaction Mix may also appear clear to cloudy upon thawing; mix thoroughly but gently before and after adding each component to the IVT reaction.
- Avoid RNase contamination by wearing gloves; working in a clean, dust-free environment; and using RNase-free tips and microcentrifuge tubes.

## Additional Materials Required

- DNA preparation kit (e.g., Thermo Scientific™ GeneJET™ Plasmid Maxi Prep Kit, Product No. K0492)
- Western immunoblot accessories for detecting expressed protein
- FITC filter-containing device to observe the expression of GFP in positive control reactions
- RNase-free microcentrifuge tubes for assembling reactions (1.5mL)
- RNase-free pipette tips
- Incubator capable of maintaining temperature at 30°C
- Optional: Shaker incubator capable of maintaining temperature at 30°C

## Protocol for Using the 1-Step Human High-Yield Mini IVT Kit

### A. Protein Expression

1. With the exception of 5X Dialysis Buffer, thaw all other reagents in the kit contents of 88890X or 88891X and maintain on ice. Thaw 5X Dialysis Buffer at 25-30°C for a maximum 15 minutes, and after making a 1X mixture, maintain the diluted buffer at room temperature.

   **Note:** Store any unused 88890X or 88891X kit components at -80°C.

   **Note:** The 5X Dialysis Buffer may appear cloudy. Mix or vortex gently. Do not centrifuge before use. Once diluted, the 1X Dialysis Buffer will become clear within minutes.

2. Combine room temperature 5X Dialysis Buffer and Nuclease-free Water (volumes per Table 1) in the provided 2.0mL microcentrifuge tube.

**Table 1. Reconstitution of the Dialysis Buffer.**

| Component | 88890 or 88891 (µL) |
|---|---|
| 5X Dialysis Buffer | 280 |
| Nuclease-free Water | 1120 |
| **Total** | **1400** |

3. Place a dialysis device inside the tube containing 1X Dialysis Buffer as shown in the Procedure Summary Section.
4. Prepare IVT reactions using Table 2. Add the reagents in the order listed into a 1.5mL RNase/DNase-free tube. Mix HeLa Lysate with Accessory Proteins and incubate for 5-10 minutes at room temperature prior to adding the remaining components. Gently mix the reaction after each reagent addition.

**Table 2. Components of the IVT reaction.**

| Component | GFP Control (µL) | Target Protein (µL) |
|---|---|---|
| HeLa Lysate | 50 | 50 |
| Accessory Proteins | 10 | 10 |
| Reaction Mix | 20 | 20 |
| pCFE-GFP DNA (0.5µg/µL) | 8 | - |
| Cloned DNA (0.5µg/µL) | - | 8 |
| Nuclease-free Water | 12 | 12 |
| **Total** | **100** | **100** |

5. Briefly centrifuge the above reactions at 10,000 × *g* for 2 minutes. A small pellet may be visible after centrifugation.
6. Transfer the supernatant into the empty dialysis device as indicated in the Procedure Summary Section, pg. 2.
7. Close the lid of the microcentrifuge tube over the dialysis device. Incubate the reaction for 6-16 hours at 30°C.

   **Note**: Although protein expression is complete within six hours for most proteins tested, incubating up to 16 hours may increase expression of some proteins. Optimal time to express each protein must be determined empirically. In addition, shaking the dialysis device containing reaction mixtures at approximately 600-900rpm during the course of incubation using a small table-top shaker incubator such as Eppendorf™ ThermoMixer™ may increase total protein yields up to 30%.

8. Resulting reactions may be stored on ice for same-day use. For long-term storage, transfer the reaction contents from the dialysis device and store separately in a 1.5mL microcentrifuge tube at -20°C or colder. Occasionally a small white precipitate may be visible, which can be removed by centrifuging the reactions at 10,000 × *g* for 2 minutes.
9. Proteins expressed using this kit may be purified using the purification guidelines provided in the Product Blog article "Choosing a vector and purification method for *in vitro* protein expression" on our website at: thermoscientific.com/pierce.

### B. Determination of Protein Expression Level

**Note:** The GFP control protein is from the copepod *Pontellina plumata*. This GFP is not reactive to antibodies generated against *Aequorea victoria* GFP (i.e., EGFP or other EGFP mutants).

**Note:** Use polyclonal antibodies to TurboGFP (Product No. PA5-22688).

1. Visualize or quantitate the GFP control protein using one of the following methods:

   **Quick visual detection:** Place the GFP reaction tubes directly under a microscope or imaging equipment containing a FITC filter; alternatively, spot a small volume (1-2µL) on a piece of plastic wrap or laboratory film and visualize with fluorescent imaging equipment.

   **Fluorescent plate reader:** Place sample directly into a white or black 96- or 384-well plate. Evaluate signal using a fluorescent plate reader at ex/em: 482/502nm. To quantitate GFP, compare the fluorescence to a recombinant GFP standard curve.

2. Visualize or quantitate non-fluorescent protein expression using one of the following methods:

   **Fast Western immunoblot analysis:** This is a quick protocol consisting of transfer and detection of proteins separated on SDS-PAGE using ultra-sensitive Thermo Scientific™ SuperSignal™ Substrate. A detailed protocol and reagents required for Western blot detection can be found at: thermoscientific.com/pierce; search using "fast western blot."

   **SDS-PAGE analysis:** Separate proteins by SDS-PAGE and stain using Thermo Scientific™ GelCode™ Blue Stain Reagent (Product No. 24590), Imperial™ Protein Stain (Product No. 24615) or PageBlue™ Protein Staining Solution (Product No. 24620) (Figure 2).

### C. Purification of IVT-expressed Proteins

Proteins expressed using this kit may be purified using the purification guidelines provided in the Product Blog article "Choosing a vector and purification method for *in vitro* protein expression" on our website at: thermoscientific.com/pierce.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| GFP not detected by fluorescence in positive control reaction | Incorrect filter set was used | The excitation/emission wavelengths of GFP are 482/502nm |
| | Lysates have become inactive | Store unused lysate in nuclease-free tubes at -80°C; do not exceed five cycles of freezing and thawing |
| No expression of target protein | Incorrect vector was used | Use the pT7CFE1-NHis-GST-CHA Vector provided in the kit to clone and express the gene of interest<br>**Note:** The 1-Step Human IVT Kits are optimized using the pCFE1 vector and its derivatives; for a complete listing, please visit our website at: thermoscientific.com/pierce |
| | HeLa Lysate, Accessory Proteins and Reaction Mix were stored at a suboptimal temperature | Store unused HeLa Lysate, Accessory Proteins and Reaction Mix in nuclease-free tubes at -80°C; do not exceed five cycles of freezing and thawing |
| | Poor-quality DNA | Ethanol precipitate the DNA to remove trace amounts of inhibitors or salts − see Additional Information Section for the recommended protocol |
| | Degradation of mRNA in the translation reaction | Maintain an RNase-free environment by wearing gloves; working in a clean, dust-free environment; and using RNase-free tips and microcentrifuge tubes |

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| | | |
|---|---|---|
| No expression of target protein | Protein was sensitive to proteases | Add Thermo Scientific™ Halt™ Protease Inhibitor Single-Use Cocktail, EDTA-free (100X) (Product No. 78425) at 0.5X to the reaction mix in Step A, 4, Table 2 |
| Low yield of target proteins | Incorrect incubation temperature | Perform reactions at 30°C |
| | Incorrect order of reagent addition | Incubate HeLa Lysate with Accessory Proteins for 5-10 minutes before adding remaining components to improve target protein expression |
| Smaller band size than predicted | Stop codons were in genes of interest | Ensure the cloned genes do not have a stop codon in the open reading frame |
| Protein appears to be degraded | Proteins were susceptible to proteases | Add Halt Protease Inhibitor Single-Use Cocktail, EDTA-free (100X) (Product No. 78425) at 0.5X to the reaction mix in Step A, 4, Table 2 |
| Larger band size than predicted | Post-translation modifications | HeLa Lysate is capable of protein post-translational modifications, including partial glycosylation and phosphorylation. Validate the presence of glycosylation by digesting a small portion of the sample with Endo H or PNGase (a loss of the higher molecular-weight bands indicates proteins were glycosylated) |
| Low protein yield after purification | Reaction scale was too small | Follow guidelines provided in the Product Blog article "Choosing a vector and purification method for *in vitro* protein expression" on our website at: thermoscientific.com/pierce |
| | | Increase reaction size |
| | Affinity tag was not accessible | Use different affinity purification for the tagged protein |
| | | Purify protein under denaturing conditions (e.g., 8M urea) using the Thermo Scientific™ HisPur™ Cobalt Purification Kit (Product No. 90090) |
| | | Reduce incubation temperature to 25°C |

## Additional Information

### A. pT7CFE1-NHis-GST-CHA Vector Cloning Sites and Sequence Features

The 1-Step High-Yield IVT Kit has been optimized using the pT7CFE1-NHis-GST-CHA cloning vector, which is designed for high-level protein expression. In addition to multiple purification tags, it contains an HRV3C cleavage site for tag removal. For a complete listing of pT7CFE1 expression vector derivatives, visit thermoscientific.com/pierce; search using "expression vectors."

**Features:**

- 10 unique restriction sites are provided in the multiple cloning site for cloning genes of interest (Figure 1)
- 5´ UTR consisting of EMCV internal ribosome entry site (IRES) required for high-level protein expression
- Poly A sequence in the 3´ region promotes mRNA stabilization and protection from nucleases
- T7 terminator ensures synthesis of accurate sized mRNA transcripts

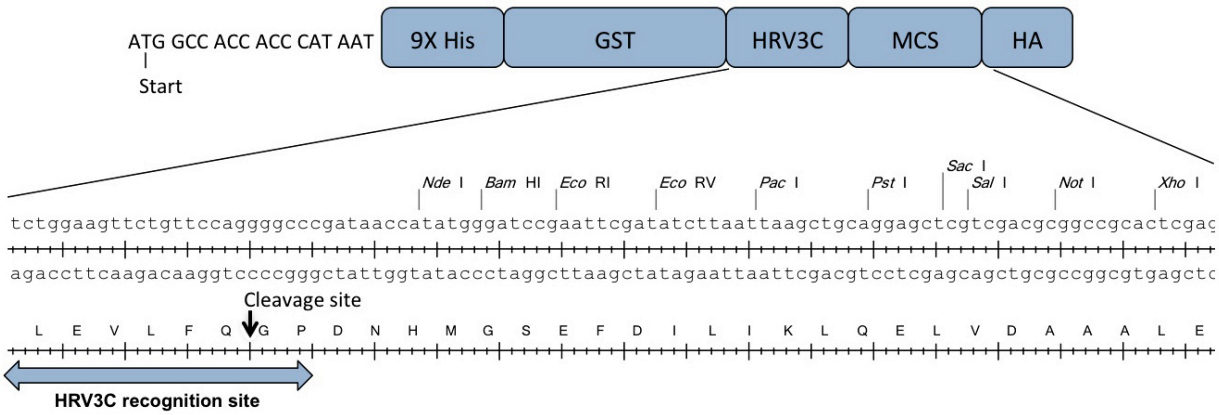


**Figure 1. The Thermo Scientific pT7CFE1-NHis-GST-CHA Vector multiple cloning site, with the exception of Msc I, is common to all of the expression vectors used in the Thermo Scientific 1-Step Human IVT Kits.** The translational start site is the ATG found upstream of the His tag region.

### B. Vector DNA Clean-up and Concentration Protocol

Prepare DNA using a standard maxi- or mini-prep protocol. To avoid compromising protein expression yield, completely remove contaminating proteins and eliminate the RNase A used in many mini-prep protocols. Perform the following steps to precipitate and, subsequently, concentrate the DNA.

1. Add 1/10 volume of 3M sodium acetate, pH 5.5 and two volumes of ethanol. Thoroughly mix the reaction and incubate at -20°C for 15 minutes.
2. Centrifuge the mixture at 14,000 × *g* for 15 minutes. Remove the supernatant and wash the pellet once with 70% ethanol.
3. Centrifuge at 14,000 × *g* for 5 minutes. Using a fine tip, remove all of the supernatant, including the residual. Air-dry the pellet for 5 minutes at room temperature.
4. Resuspend the pellet in nuclease-free water before measuring the DNA concentration. DNA templates may be stored in a Tris-based buffer. It is not necessary to linearize the plasmid DNA before using it in transcription reactions.

### C. Expression-ready Clones for Use with the 1-Step Human High-Yield IVT Kit

- Custom cloning service; please visit thermoscientific.com/pierce; search using “cloning service.”
- The pANT7 vector library from the ASU Biodesign Institute DNASU Plasmid Repository is compatible with the 1-Step Human High-Yield IVT Kit. Visit http://dnasu.asu.edu/DNASU/Home.jsp for information and ordering. Under advanced search options choose “pANT7” for vector selection.
- PCR templates: see Tech Tip #72: PCR protocol for generating optimized templates for Pierce™ Human IVT Expression Kits on our website.

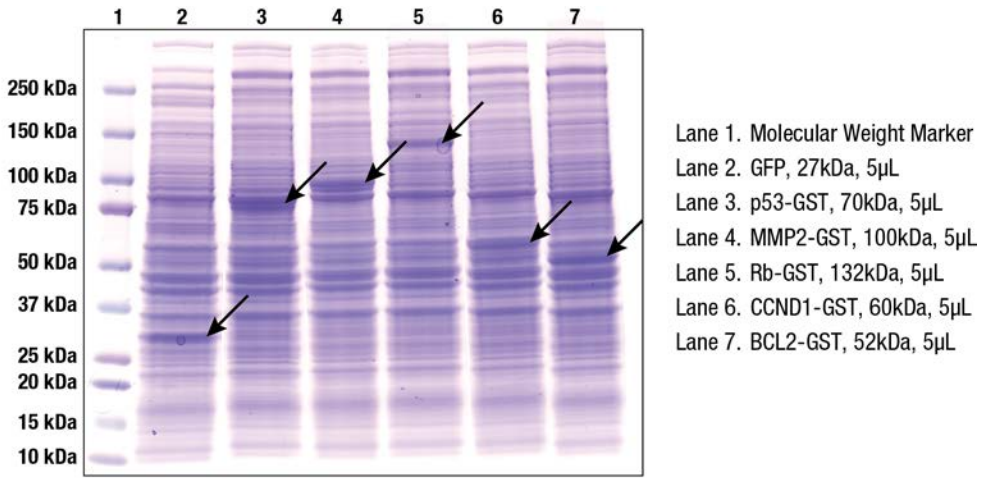


**Figure 2. Expression of coomassie-stainable proteins using the Thermo Scientific 1-Step Human High-Yield IVT Kit.** Five expression-ready clones (pANT7-based vector) obtained from the DNASU Plasmid Repository were used to express the GST-fusion proteins listed in Lanes 3-7. Lane 2 shows expression of the control pCFE-GFP plasmid. Reaction mixtures of 5µL were separated by SDS-PAGE and stained with GelCode Blue Stain Reagent. Arrows indicate the positions of expressed proteins.

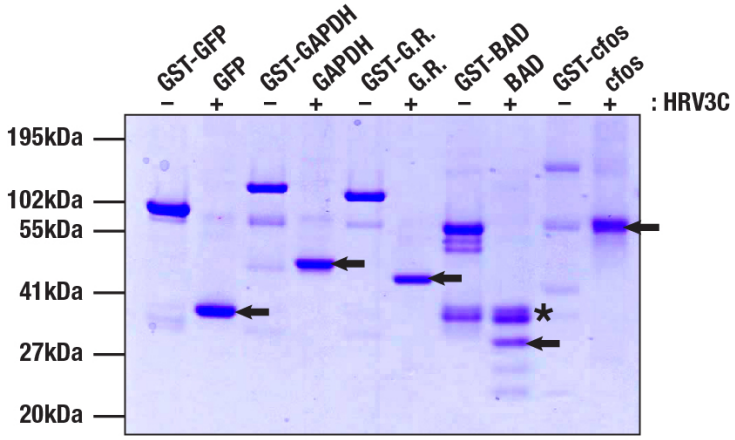


**Figure 3. Purification of N-terminal GST fusion proteins with immobilized glutathione.** Purification of either GST-fused proteins or untagged protein was performed as described with 10mM glutathione or HRV3C protease, respectively, using instructions provided in the Product Blog article "Choosing a vector and purification method for *in vitro* protein expression" on our website at: thermoscientific.com/pierce. The additional bands denoted with a * found with the purification of Bad are 14-3-3 proteins, which co-elute with Bad. Protein identification was verified by mass spectrometry (data not shown).

## Related Thermo Scientific Products

| | |
|---|---|
| **88859-71** | **pT7CFE1-based Expression Vectors** |
| **88899** | **Recombinant GFP Protein** |
| **88881-2** | **1-Step Human Coupled IVT Kit – DNA** |
| **88892** | **1-Step Human High-Yield Maxi IVT Kit** |
| **MA121315** | **Mouse anti-6x-His Epitope Tag Monoclonal Antibody (HIS.H8)** |

| | |
|---|---|
| **26183** | **Mouse anti-HA Monoclonal Antibody (2-2.2.14)** |
| **MA4004** | **Mouse anti-Glutathione S-transferase Monoclonal Antibody (8-326)** |
| **35035** | **Pierce Fast Semi-Dry Transfer Buffer (10X),** 500mL |
| **88217** | **Pierce Fast Semi-Dry Blotter** |
| **35050** | **Pierce Fast Western Blot Kit, ECL Substrate** |
| **88221** | **HisPur Ni-NTA Resin,** see our website for all related products |
| **89964** | **HisPur Cobalt Resin,** see our website for all related products |
| **16100** | **Pierce Glutathione Agarose,** see our website for all related products |
| **26182** | **Pierce Anti-HA Agarose,** see our website for all related products |
| **K0492** | **GeneJET Plasmid Maxiprep Kit,** see thermoscientific.com/onebio |
| **PA5-22688** | **Anti-TurboGFP Polyclonal Antibody** |
| **88836-7** | **Pierce Anti-HA Magnetic Beads** |
| **88821-2** | **Pierce Glutathione Magnetic Beads** |
| **88831-2** | **HisPur Ni-NTA Magnetic Beads** |

## General References


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