INSTRUCTIONS

# 1-Step CHO High-Yield IVT Trial Kit

## 88893

2595.0

| Number | Description |
|---|---|
| **88893** | **1-Step CHO High-Yield IVT Trial Kit,** contains sufficient reagents to perform 2 reactions (100µL each) |

| Kit Contents | Cap Color | 88893X |
|---|---|---|
| CHO Lysate | Silver seal | 1 lyophilized vial |
| Accessory Proteins | Green | 25µL |
| 5X Reaction Mix | Yellow | 40µL |
| 4X Dialysis Buffer | Clear | 700µL |
| Positive Control DNA: pCFE-GFP (0.5µg/µL, 10µg) | Clear | 20µL |
| pT7CFE1-NHis-GST-CHA Expression Vector (0.5µg/µL, 10µg) | Clear | 20µL |

| Kit Contents | 88893Y |
|---|---|
| Microdialysis Device | 2 each |
| Nuclease-free Water | 50mL |

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

**Storage:** Upon receipt store 88893X at -80°C and 88893Y at room temperature. 88893X is shipped with dry ice. 88893Y is shipped at ambient temperature.

## Table of Contents




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## Introduction

The Thermo Scientific™ 1-Step CHO High-Yield IVT Trial Kit is a mammalian *in vitro* translation (IVT) system based on CHO cell lysates. The kit contains 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, synthesize proteins faster and label protein 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 CHO *in vitro* protein expression system.

## Procedure Summary

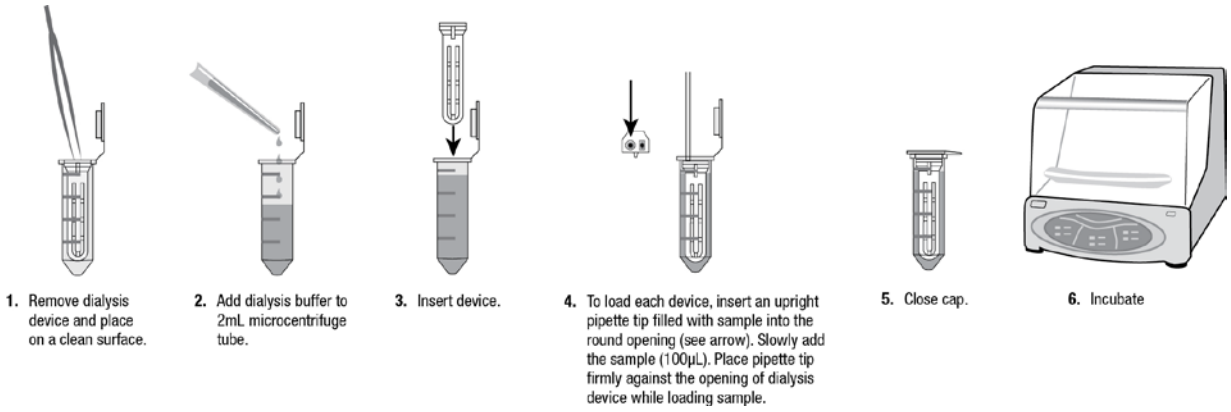


**Figure 1. The Thermo Scientific 1-Step CHO High-Yield IVT Trial Kit protocol.**

## 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.**
- CHO Lysate is provided as a lyophilized lysate. Reconstitute contents on ice, as recommended in the Protocol Section. Aliquot if necessary and quickly store at -80°C. All components of the kit are stable for up to five freeze-thaw cycles as long as the contents are stored at -80°C immediately after use.
- 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. Undiluted 4X Dialysis Buffer may appear cloudy; mix well before and after dispensing.
- 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. K0503 or K0492)
- Western immunoblot accessories for detecting expressed protein
- FITC filter-containing device to observe the expression of GFP in positive-control reactions
- 1.5mL RNase-free microcentrifuge tubes for assembling reactions
- RNase-free pipette tips
- Optional: Shaker/incubator capable of maintaining temperature at 30°C

## Protocol for Using the 1-Step CHO High-Yield IVT Trial Kit

### A. Protein Expression

1. With the exception of 4X Dialysis Buffer, thaw all other reagents in the kit contents of 88893X and maintain on ice. Thaw 4X Dialysis Buffer at 25-30°C for a maximum of 15 minutes and, after making a 1X mixture, maintain the diluted buffer at room temperature.

   **Note:** Store any unused 88893X kit components at -80°C.

   **Note:** The 4X 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. Remove dialysis device as shown in Step 1 of Procedure Summary. Combine room temperature 4X Dialysis Buffer and Nuclease-free Water (volumes per Table 1) in the provided 2mL microcentrifuge tube.

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

| Component | µL |
|---|---|
| 4X Dialysis Buffer | 350 |
| Nuclease-free Water | 1050 |
| **Total** | **1400** |

3. Place the dialysis device inside the tube containing 1X Dialysis Buffer as shown in the Procedure Summary Section. Confirm dialysis membrane is completely immersed. Incubate at 30°C for 30 minutes.
4. **Reconstitution of the lyophilized CHO Lysate vial:** Add 110µL of Nuclease-free Water provided in the kit to the lyophilized vial. Let it stand for 2-3 minutes and then slowly pipette up and down the contents of the tube at least 5 times to completely reconstitute the lysate. Store at -80°C any unused portion of the CHO Lysate.
5. Prepare IVT reactions using Table 2. Add the reagents in the order listed into a 1.5mL RNase/DNase-free tube at room temperature. Mix the CHO Lysate with Accessory Proteins and incubate for 5-10 minutes at room temperature before 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) |
|---|---|---|
| CHO Lysate | 50 | 50 |
| Accessory Proteins | 10 | 10 |
| 5X 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** |

6. Transfer the supernatant into the empty dialysis device as described in the Procedure Summary Section.
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 6 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.

   **Note:** Shaking the dialysis device containing the reaction mixture at ~600-900rpm during incubation may increase total protein yields up to 30%. Use a small table-top shaker/incubator such as an Eppendorf™ Thermomixer™ Shaker.

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 (ex/em: 482/502nm); 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), Thermo Scientific™ Imperial™ Protein Stain (Product No. 24615) or Thermo Scientific™ PageBlue™ Protein Staining Solution (Product No. 24620) (Figure 2).

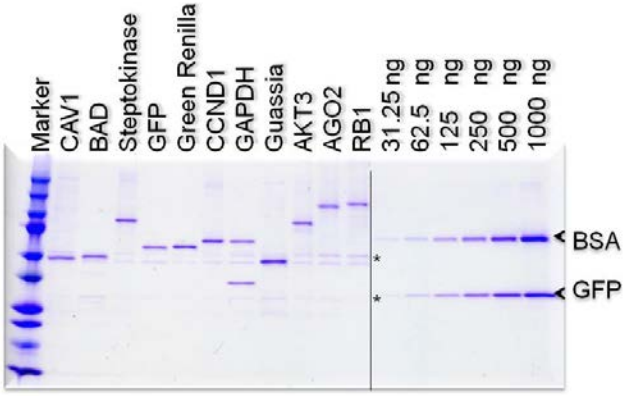


**Figure 2. Purification of N-terminal GST fusion proteins with immobilized glutathione.** Genes cloned into pT7CFE1-NHis-GST-CHA were used to express GST-fusion proteins for 6 hours following the procedure described above. Purification of GST-fused proteins was performed as described with 50mM glutathione 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 * and found with the purified proteins were previously identified as cellular proteins eEF1G and GSTM3 by mass spectrometry. These proteins are known to bind to the glutathione column and co-elute with GST-tagged proteins. Approximately 500ng of each of the purified proteins were separated by SDS-PAGE and stained using the Thermo Scientific™ Pierce™ Power Stainer (Product No. 22833).

### 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 used | The excitation/emission wavelengths of GFP are 482/502nm |
| | Lysates became 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 used | Use cloning vector pT7CFE1-NHis-GST-CHA provided in the kit to clone and express the gene of interest<br>**Note:** The 1-Step CHO High-Yield IVT Kits are optimized using the pCFE1 vector and its derivatives; for a complete listing, please visit thermoscientific.com/pierce. See also Section C in Additional Information for the readily available clone collection |
| | CHO Lysate, Accessory Proteins and Reaction Mix were stored at a suboptimal temperature | Store unused CHO 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 the 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 |
| | 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 Table 2 |
| Low yield of target proteins | Incorrect incubation temperature | Perform reactions at 30°C |
| | Incorrect order of reagent addition | Incubate CHO 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 Table 2 |
| 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 |
| Low protein yield after purification | 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) |

## Additional Information

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

The 1-Step CHO High-Yield IVT Kits have 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 HRV 3C 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

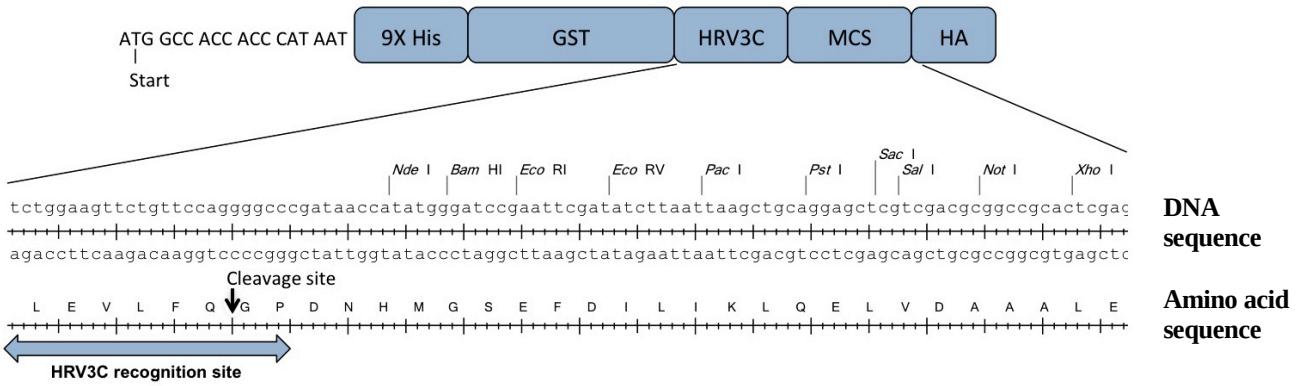


**Figure 1. The Thermo Scientific pT7CFE1-NHis-GST-CHA Vector multiple cloning site, with the exception of Msc 1, is common to all of the expression vectors used in the Thermo Scientific 1-Step High-Yield CHO IVT Kits.** The translational start site is the ATG found upstream of the His tag region.

### 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 use.

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

- Custom cloning service; please visit thermoscientific.com/pierce and search for "cloning service."
- The pANT7 vector library from the ASU Biodesign Institute DNASU Plasmid Repository is compatible with our 1-Step CHO 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 *In Vitro* Expression Kits on our website.

## Related Thermo Scientific Products

| | |
|---|---|
| **88894** | **1-Step CHO High-Yield Maxi IVT Kit** |
| **88859-71** | **pT7CFE1-based Expression Vectors** |
| **88899** | **Recombinant GFP Protein** |
| **88881-2** | **1-Step Human Coupled IVT Kits — DNA** |
| **88890-2** | **1-Step Human High Yield IVT Kits** |
| **MA4004** | **Mouse anti-Glutathione S-transferase Monoclonal Antibody (8-326)** |
| **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)** |
| **PA5-22688** | **Anti-TurboGFP Polyclonal Antibody** |
| **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 |
| **88836-7** | **Pierce™ Anti-HA Magnetic Beads** |
| **88821-2** | **Pierce™ Glutathione Magnetic Beads** |
| **88831-2** | **HisPur™ Ni-NTA Magnetic Beads** |
| **90410-1** | **Low Protein Binding Microcentrifuge Tubes, 2.0mL** |

### General References


Imataka, H., *et al.* (2009). Advantages of CHO cell-derived, cell-free protein synthesis systems (Japanese). *Seikagaku* **81(4)**:303-7.

Brödel, A.K., Sonnabend, A., Kubick, S. (2014) Cell-free protein expression based on extracts from CHO cells. *Biotechnol Bioeng* **111(1)**:25-36.

Kozak, M. (1983). Comparison of initiation of protein synthesis in prokaryotes, eukaryotes and organelles. *Microbiol Rev* **47(1)**:1-45.

Kozak, M. (2005). Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene* **361**:13-37.



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