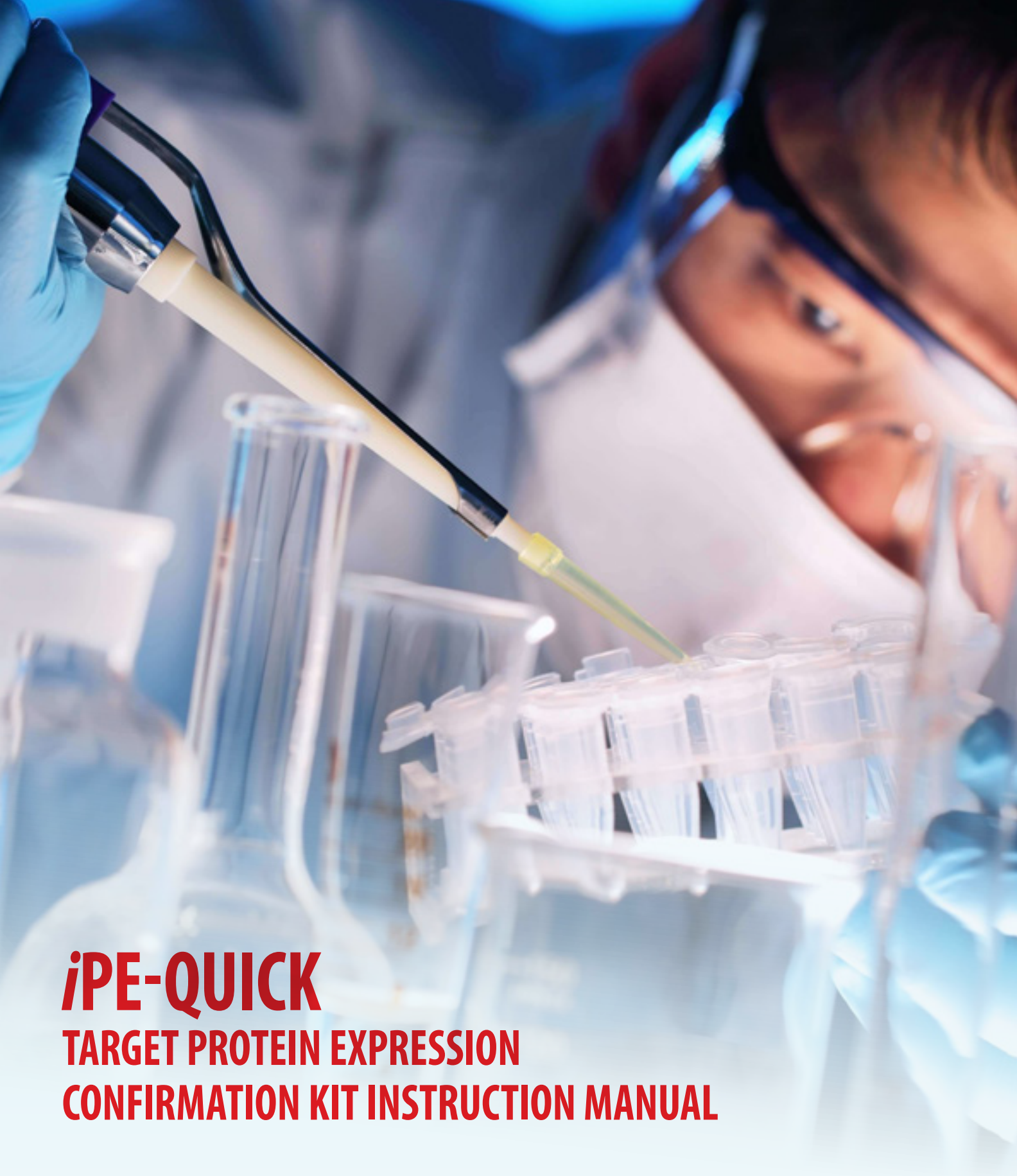


*i*PE-QUICK

TARGET PROTEIN EXPRESSION CONFIRMATION KIT INSTRUCTION MANUAL

ISOTEC®

*i*PE-QUICK

Target Protein Expression Confirmation Kit

Use this product to confirm target protein expression prior to stable isotope labeled protein synthesis with the *in vitro* Protein Expression (*i*PE) Kit (**Prod. No.** 767816) sold separately.

Read the entire instruction manual before using this product.

Introduction

Thank you for your purchase of the target protein expression confirmation kit, *i*PE-Quick.

This kit has been developed under license from RIKEN, incorporating its proprietary, advanced cell-free protein synthesizing technology into a kit.

The *i*PE-Quick Kit is intended for the advanced confirmation of target protein expression utilizing *E.coli* extract before the use of the *i*PE Kit (**Prod. No.** 767816). Unlabeled amino acids are added to allow easy and efficient protein expression by adding circular or linear DNA as the template DNA, which enables transcription of mRNA with T7 RNA polymerase.

Safety Precautions

Review and adhere to all guidelines to avoid potential injury.

- This kit is for experimental and/or research use only. Do not use this kit, the protein, or any other components obtained from this kit for medical care and/or clinical diagnosis of humans or animals and/or add them to beverages or food.
- This kit is intended to be used by experts with experience in general biochemical experiments as well as micropipette operations. Personnel without such experience must not use this product.
- Wear proper safety goggles, gloves, lab coat, and other protective gear when handling the product. If the solution comes into contact with the eyes and/or skin, wash it away using clean running water. If any inflammation occurs, seek medical attention immediately.
- Please note that we assume no liability for any problems that might occur from any use of this product not authorized by this manual.

Storage

- Store at -80 ± 2 ˚C
- If the kit is stored at temperatures significantly deviating from -80 ˚C, the protein synthesis performance will be significantly lowered.
- Do not refreeze or store once the kit has been thawed. The synthesis performance will be significantly lowered.

Warning: Do not handle this kit with bare hands. It is stored at -80 ˚C creating a risk of frostbite.

Kit Contents

- Quick Solution* – 1 mL in a 1.5 mL tube (equivalent to 50 µL reaction × 20 times)
- Control DNA** (pUC-CAT) – 50 µL in a 1.5 mL tube

* The amino acids contained in this kit are not labeled with stable isotopes.

** Expresses chloramphenicol acetyl transferase (CAT).

Composition of Quick Solution

Quick Solution		
E.coli cell extract	NTPs	Natural Abundance Amino Acids
Creatine Kinase	HEPES-KOH (pH 7.5)	Ammonium Acetate
T7 RNA Polymerase	Polyethylene Glycol	Magnesium Acetate
tRNA	D-Glutamate	Creatine Phosphate
DTT	cAMP	Folinic Acid

Template DNA

This kit does not include template DNA. For protein expression, template DNA for the target protein containing T7 promoter and T7 terminator that enables transcription of mRNA with T7 RNA polymerase is required, as shown in the figure below.

Prepare template DNA for the target protein before using the kit. Use the following concentrations and amounts as a guideline.

- If circular DNA is used as template DNA: At least 10 µL (30 µg/mL) is required.
- If linear DNA is used as template DNA: At least 10 µL (60 µg/mL) is required.

Note: Optimal concentration may vary depending on the molecular weight of the template DNA. We recommend that the template DNA concentration be optimized as required. Since the template DNA design has a significant influence on the amount of expressed protein, it is necessary to examine optimization of the expression range and amino-acid sequence according to the type of the target protein.

Devices, Reagents, etc. Required for Using this Kit

1. Template DNA
2. Sterilized 0.6 mL sample tubes for the number of samples (up to 20 tubes)
3. Aluminum block heater or water bath (37 ˚C)
4. Micro pipettes
5. Table-top centrifuge
6. Ice bath

Protein Synthesis Operation

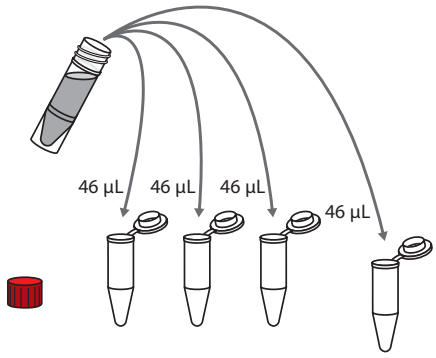
1. Before use, check all packages and containers to ensure that there has not been any damage incurred to the product.
2. Dissolve the template DNA in distilled water or buffer.
3. Remove the kit from the freezer and open the package.
4. Thaw the control DNA in an ice bath.
5. Thaw the quick solution in a water bath set to 37 °C for three minutes.

Notes:

The thawing time given is a guideline. Adjust the time as required.

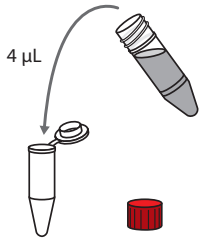
Use the solution immediately after thawing. The performance will be significantly lowered if the solution is left for an extended period of time.

6. Immediately after thawing, spin down the quick solution using a table-top centrifuge and mix the solution using a micropipette.
7. Dispense 46 µL of quick solution into each 0.6 mL sample tube placed in an ice bath for cooling.



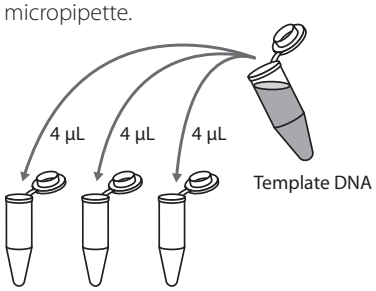
An example of 3 sample tubes and 1 control tube

8. Add 4 µL of the control DNA (pUC-CAT) to a 0.6 mL sample tube as a control solution and mix it with a micropipette.



Tube for control (CAT)

9. Add 4 µL of the template DNA to each 0.6 mL sample tube and mix it with a micropipette.



10. Incubate the samples at 37 °C for one hour.

Notes: The protein synthesis is completed in approximately one hour. Even if the solution is incubated for a longer period of time, the reaction will not continue.

The *i*PE-Quick kit has been optimized at 37 °C however it has been shown to work in the temperature range of 25-37 °C. Reaction can be run at optimal temperature for the DNA template but may have reduced expression.

11. Cool the tubes in an ice bath to terminate the reaction.
12. Centrifuge the solution at 4 °C and 15,000 RPM for five minutes. The expressed soluble protein will be contained in the supernatant.

Note: If the solution is left in this state for an extended period of time, the expressed protein may precipitate or decompose. Confirm the protein expression immediately with an appropriate method.

Observation of expression by SDS-PAGE

An acetone precipitation is required in order to remove the polyethylene glycol from the reaction mixture before SDS-PAGE. Polyethylene glycol may disturb the protein bands on SDS-PAGE.

Transfer the 3 µL of Reaction solution supernatant into a 0.6 mL Eppendorf tube (Total fraction). Centrifuge the Reaction solution at 12,000 rpm for five minutes at 4 °C and transfer the 3 µL of supernatant into another 0.6 mL Eppendorf tube (Soluble fraction). Add both 27 µL of distilled water and 600 µL of cold acetone to each of the Total fraction and Soluble fraction. Let both tubes stand on ice for five minutes, then centrifuge at 12,000 rpm for five minutes at 4 °C, and discard both supernatants. Dry the pellets in air and add 30 µL of 1 × SDS-PAGE sample buffer into each fraction. For a mini slab gel, apply 3-6 µL per well.

Disposal

The solution of this kit, along with all containers, devices, etc. that may have come into contact with the solution, must be sterilized using an autoclave or similar process, then must be discarded in accordance with local regulations. Refer to the MSDS document for additional details. Protein and other products obtained by this kit must be discarded properly and are the responsibility of the customer.

Caution: *E. coli* bacteria (non-recombinant) may remain within the kit.

CAT Activity Measurement

The amount of CAT protein synthesized by the attached control DNA can be quantified by a colorimetric method such as CAT activity assay (Shaw, 1975).

Typically, around 600 µg/mL active protein is obtained from the reaction solution.

Troubleshooting

If protein does not express, the expressed protein amount is small, or for other issues refer to the following table.

Possible Cause	Recommended Action
Kit performance deterioration	1. Check that the expiration date for use written on the product label is not exceeded. 2. Check that the product was stored at the proper temperature.
Inappropriate template DNA	Check that the primary structure, sequence, and concentration of template DNA are correct. If the purity of DNA is low, only a small amount of protein may be expressed. Check the purity of the template DNA used with UV measurement. In general, an absorbance ratio of A260/A280 ≥ 1.8 is considered a requirement for high purity DNA. If you re-purify template DNA, please use commercially available purification kits or perform purification via phenol/chloroform extraction and ethanol precipitation.
Problems due to protein property	Some proteins may easily decompose and/or precipitate during the reaction. The solubility may be improved by inserting tag sequences. Note that the amount of expressed protein varies greatly by the expression area and amino acid composition at terminal sequence; make sure to optimize the construct according to the protein.

Warranty

The kit is delivered frozen on dry ice. If there is no dry ice remaining in the delivery box at the time of arrival, or if there is damage to the package and/or solution has leaked, the quality of the components in this kit may be compromised. Contact us immediately if any of these delivery issues have occurred. The warranty remains in effect until the expiration date marked on the product label.

Disclaimer

We assume no liability for the following even within the warranty period:

- Defects caused by improper storage and/or improper usage.
- Defects unrelated to the performance of this kit.
- Protein expression may decrease due to various factors other than the performance of the kit. For this reason, we do not guarantee the expression of protein.
- Please note that we assume no liability for passive damages due to defects of this kit or damages due to products obtained using this kit and similar.

References

1. Kigawa T. et. al., *Cell-free Protein Synthesis Methods and Protocols* (Spirin, A. S. & Swartz. J. R., eds.), 83-97 (2007)
2. Matsuda T, et. al., *J Biomol NMR*, **37** (3) 225-229 (2007)
3. Shaw. W. V., *Methods in Enzymology*, **43**, 737-755 (1975)
4. Seki E. et. al., *Anal. Biochem.*, **377**, 156-161 (2008)
5. Yabuki T. et. al., *J. Struct. Func. Genomics*, **8** (4), 173-191 (2007)
6. Yokoyama J. et. al., *Anal. Biochem.*, **411**, 223-229 (2011)

ISOTEC® Contact Information

We take all possible measures to ensure the correctness of all information contained in this manual. Should you have any questions or comments, notice any omissions, etc., contact us.

ISOTEC Stable Isotopes Technical Service
(937) 859-1808
(800) 448-9760 (US and Canada)
Email: isosales@sial.com

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Order/Customer Service: sigma-aldrich.com/order
Technical Service: sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® safcglobal@sial.com
Safety-related Information: sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

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