Sigma-Aldrich®
Lab & Production Materials

Instruction Manual

In Vitro Protein Expression Kit

Stable Isotope Labeling Protein Synthesis Kit

Prod. No. 905089

Introduction

Thank you for your purchase of the In Vitro Protein Expression Kit (iPE Kit).

This kit has been developed under license from RIKEN, incorporating their proprietary, advanced cell-free protein synthesizing technology into a kit dedicated to stable isotope labeling. It dramatically reduces isotope dilution compared to conventional products.

This is a protein synthesis system that utilizes *E. coli* cell extract. It allows easy and efficient protein expression by adding circular or linear DNA as the template DNA, which enables transcription of mRNA with T7 RNA polymerase.

It is important to confirm the expression of target protein by first using the separately sold iPE-Quick Kit (Prod. No. 767824).

It is important to read this instruction manual thoroughly in order to understand proper handling methods.

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

- Internal solution – 775 µL (one 1.5 mL tube)
- External solution – 8.25 mL (one 25 mL tube)
- Dialysis membrane cup
- Control DNA pUC-CAT (50 uL) and amino acid mixture (natural abundance, 1 mL) also included.

Note: Stable isotope labeled amino acid mixtures are not included in this kit. Please use the separately sold stable isotope labeled amino acids mixtures available from ISOTEC® Stable Isotopes (Prod. No. 767964, 767972, 771031). Aldrich.com/isotec

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Composition of Kit Components

Internal Solution		
E. coli cell extract	NTPs	Folinic Acid
Creatine Kinase	HEPES-KOH (pH 7.5)	Ammonium Acetate
T7 RNA Polymerase	Polyethylene Glycol	Magnesium Acetate
tRNA	D-Glutamate	Creatine Phosphate
DTT	0.05% NaN_3	cAMP

External Solution		
NTPs	Folinic Acid	HEPES-KOH (pH 7.5)
Ammonium Acetate	Polyethylene Glycol	Magnesium Acetate
D-Glutamate	Creatine Phosphate	DTT
0.05% NaN_3	cAMP	

Caution: Solutions contain 0.05% sodium azide (NaN3). Sodium azide is designated as a poisonous substance in the Japanese Poisonous and Deleterious Substances Control Act when reaching 0.1% levels. Handle and discard with care.

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 50 µL (50 µg/mL) is required.
- If linear DNA is used as template DNA: At least 50 µL (100 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

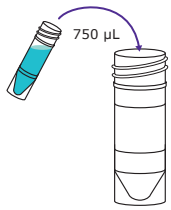
1. Stable isotope labeled amino acid mixture solution for iPE kit (Prod. No. 767964, 767972, 771031). 1 × 1 mL unit containing the labeling pattern of choice is required.
2. Template DNA
3. Water bath (30 °C)
4. Micropipette
5. Table-top centrifuge
6. Ice bath
7. Constant temperature box (30 °C)
8. Sterile water

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. Open the aluminum package containing the dialysis cup and remove the dialysis cup. Pour 1 mL of sterile water into the dialysis cup and wait for approximately 30 seconds. Check for leaks and remove the distilled water from the dialysis cup with a micropipette so that a membrane does not break.
3. Open the kit, remove only the External solution from the freezer and thaw it for 15 minutes in a water bath set to 30 °C. Similarly, place the template DNA and amino acids mixture solution into an ice bath to thaw.

   Note: The thawing time given is a guideline; adjust the time as required.
4. Place the thawed External solution in an ice bath.
5. Make sure the amino acid mixture is in good suspension. Add 750 µL of the amino acid mixture solution to the External solution container. Mix thoroughly with a micropipette.

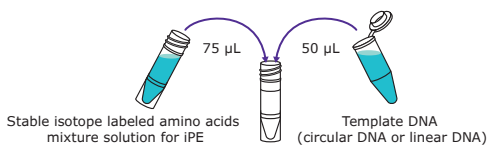
   Notes: Some stable isotope amino acid mixtures may contain precipitates. This is a normal occurrence as several amino acids have low solubility.



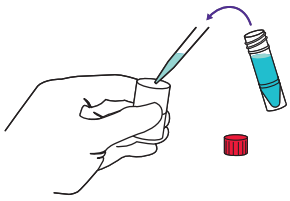
6. Remove Internal solution from the freezer and thaw for three minutes in a water bath set to 30 °C.

   Note: The thawing time given is a guideline; adjust the time as required.
7. After thawing the Internal solution, immediately spin it down using a table-top centrifuge.

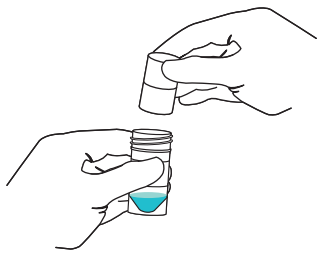
   Note: The performance may be lowered if the solution is left for an extended period of time.
8. Add 75 µL of stable isotope labeled amino acid mixture solution to the Internal solution. Add 50 µL of the template DNA, and mix thoroughly with a micropipette.



9. Dispense the Internal solution into the dialysis cup slowly so that it does not froth or produce any damage to the membrane.



10. Place the dialysis cup into the External solution container cautiously and slowly so that no air bubbles are locked between the External solution surface and the membrane.



11. Place the cap onto the External solution container. Place the External solution container vertically inside the constant temperature box. Set the constant temperature box to 30 °C.

12. Let the reaction sit for 4 to 16 hours.

    Note: Adjust the synthesis reaction time according to the type of target protein.

13. Remove the dialysis cup from the External solution container. Collect the Reaction solution from the dialysis cup and place it into a 1.5 mL microtube using a micropipette. Place the microtube into an ice bath to end the reaction.

14. Separate the collected Reaction solution by centrifuge at 4 °C and 15,000 rpm for five minutes to obtain a solution containing the target soluble protein 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. Purify and use 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 X 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.

Troubleshooting

Protein Purification Method

The target protein expressed in the collected reaction solution can be purified by a method appropriate for the protein. In the case of absorption of a protein in a resin, it is recommended that the collected reaction solution first be diluted to approximately five-fold the original volume with the buffer used for resin equilibration. 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 is not exceeded. 2. Check that the product was stored at the proper temperature.
Inappropriate template DNA	1. Confirm the protein expression using iPE-Quick (Prod. No. 767824). 2. Check that the primary structure, sequence, and concentration of the template DNA are correct. If the purity of DNA is low, only a small amount of protein may be expressed. Re-purify the template DNA using commercially available purification kits.
Problems due to protein property	Some proteins may easily decompose and/or precipitate during the reaction. Collect protein after a short reaction time (approx. four hours) and confirm the amount of expression again. The property may be improved by varying the sequence or redesigning the construct.

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. Yabuki T. et. al., J. Struct. Func. Genomics, 8 (4), 173-191 (2007)
4. Seki E. et. al., Anal. Biochem., 377, 156-161 (2008)
5. 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@milliporesigma.com**

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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