1st edition: 2023.7

Cat. No. A240-0305

Protein synthesis kit

Musaibo kun ® N 1000 SS

Operating instructions

This product is for unlabeled protein synthesis. For the synthesis of stable isotope-labeled proteins, use "Musaibo kun®SI SS" or "Musaibo kun® SI SS (without PEG)".
Before use, please read this manual thoroughly to understand proper handling methods.

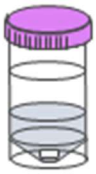
- For improvement purposes, the specifications, designs, text, etc. contained in this manual are subject to change without notice.
- We take all possible measures to ensure the correctness of all information contained in this manual. Should you have any questions, comments or notice about any omissions, please let us know at the following email address.

Customer Support E-mail: Isotope.TNS@tn-sanso.co.jp

TAIYO NIPPON SANSO

Quick protocol

Musaibo kun® N 1000 SS


Dialysis solution premix


Reaction solution premix

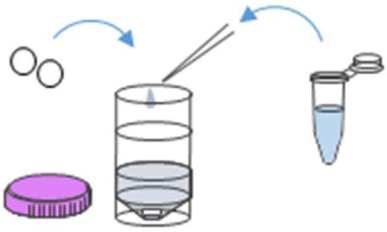

Control DNA (pUC-BAP)

Supplied by user


Tempate DNA


Sterlized water

1. Preparation of dialysis solution



Thaw the dialysis solution premix in a 30˚C water bath. (approximately 15 minutes)

Add Add 2 mL of sterilized water and 2 shaking ball into the dialysis solution premix.
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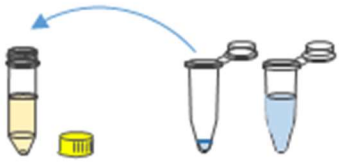
2. Preparation of reaction solution

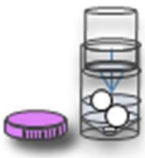


Thew the reaction solution premix in a 30˚C water bath. (approximately 1 minute)

Add 50 µL of the 100 ng/ µL template DNA and 150 µL of sterilized water into the reaction solution premix.

3. Set up the device

Gentry transfer the reaction solution into the dialysis cup.



Float the dialysis cup on the dialysis solution gently. Make sure no air bubbles trapped under the dialysis membrane, then close the lid.

4. Protein synthesis

Incubate 30˚C for 6 hours with gentle circular shaking.
Make sure the upper shaker ball is rotating slowly.

1. Introduction

Thank you very much for purchasing the Protein Synthesis Kit "Musaibo kun® N 1000 SS". This kit is developed under license from RIKEN, incorporating its proprietary, advanced cell-free protein synthesizing technology into a kit.

"Musaibo kun® N 1000 SS" is a protein synthesis system kit using *Escherichia coli* cell extract. This kit allows you to easily synthesize target proteins up to several milligrams per each reaction using the attached dialysis device. The synthesized protein can be directly processed for purification.

2. Safety Precautions

This product is for research use only. Do not use this kit, the protein, or any other components obtained from this kit for medical care and/or clinical diagnoses of humans and animals and/or add them to beverages or foods.

This kit is intended to be used by expert 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.

The following caution box indicates items where special attention is required to ensure safe usage. Make sure to observe points indicated by this caution.

Caution	Risk of occurrence of minor injury or minor physical damage in case of improper handling

3. Kit contents

- Reaction solution premix (for 1 mL reaction solution) 1
- Dialysis solution premix (for 10 mL dialysis solution) 1
- Control DNA (100 ng/μL pUC-BAP, 50 μL) * 1
  The plasmid expresses *E. coli* alkaline phosphatase
- Shaker ball 2
- Dialysis device (MWCO: 15,000) 1

4. Storage

Store at -80˚C ±2˚C

If the kit is stored at temperatures significantly deviating from -80˚C, the protein synthesis performance is lowered.

When transporting or temporarily storing, use a container such as Styrofoam containing dry ice.

Once thawed, the reaction mixture premix should be quickly placed on ice and subdivided into the required amount, quickly frozen in liquid nitrogen or powdered dry ice, and stored at -80˚C. Other solutions should be similarly aliquoted and stored at -30˚C or below. Please use up the subdivided solution when you use it next time. The protein expression efficiency will be decreased by repeated freeze-thaw cycles.

5. Main Components

Reaction solution premix

E. coli cell extract	Folinic acid	NTP
Magnesium acetate	Creatine phosphate	HEPES
T7 RNA polymerase	Amino acids (20 species)	tRNA
Sodium azide	Creatine kinase	DsbC
GSSG	L-Potassium glutamate	

Dialysis solution premix

Magnesium acetate	Creatine phosphate	NTP
Potassium acetate	HEPES	Sodium azide
Amino acids (20 species)	Folinic acid	GSSG
L-Potassium glutamate		

Contains 0.05% sodium azide, a designated poisonous substance under the Deleterious Substances and Deleterious Substances Control Law in Japan. Since the content is 0.1% or less, the above does not apply, but please be careful in handling and disposal.

6. Disposal

Caution

E. coli bacteria (not genetically-modified) may remain within the kit. The solution of this kit, along with containers, devices, etc. that the solution contact with, must be sterilized using an autoclave or similar and then discarded in accordance with local regulations, etc. upon referring to "5. Main Components" and the attached MSDS.

Protein and other products obtained by this kit must be used and discarded properly at the customer's responsibility.

7. Warranty

Warranty period

Please use this kit within the expiration date attached to the instruction manual.

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 out, the quality may have been lowered. Please contact us; we will replace the product free of charge.

Disclaimer

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

1) Defects caused by improper storage and/or improper usage

2) Defects unrelated to the performance of this kit

Protein synthesis activity may decrease due to various factors other than the performance of the kit. For this reason, we do not guarantee the expression of a 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.

8. Template DNA

To express protein using this kit, please prepare a template DNA containing the essential components for mRNA transcription by T7 RNA polymerase and translation. These components include the T7 promoter, the T7 terminator, and the ribosome binding site (RBS), target gene, as illustrated in the diagram below. Please ensure that all necessary elements are included in the template DNA to facilitate successful protein expression.

9. Protein Synthesis Procedure

Caution	Do not handle this kit with bare hands. Since the kit is stored at -80°C, there is risk of frostbite. Make sure to wear proper safety goggles, gloves, lab coat, and other protective gear when handling this kit. If the solution of the kit comes into contact with the eyes and/or skin, it may cause inflammation.

Devices, Reagents, etc. Prepared by User

Prepare the following items before using the kit:

1) Template DNA (Circular DNA recommended)

- At least 50 μL of template DNA (50 ng/μL or more). Recommended final concentration in the reaction solution is between 2-20 ng/μL.
- Please refer to our website to see detail about how to prepare the template DNA.

2) Sterilized distilled water 3) Micropipette

4) Desktop centrifuge 5) Water bath (30℃)

6) Incubation shaker (30 ˚C, rotation) 7) Ice bath

8) Sample tube

Preparation

① Check leak of the dialysis cup:

*Be sure to check before thawing the kit.

1) Remove the dialysis cup from the container lid.

2) Fill the dialysis cup with 1 mL of distilled water and confirm no water leakage from the bottom dialysis membrane.

3) Remove distilled water from the dialysis cup with a micropipette so as not to damage the dialysis membrane.

*If the dialysis cup is found to be leaking, we will replace it free of charge.

② Thaw the dialysis solution premix in a 30˚C water bath. (approximately 15 minutes) and leave at room temperature after thawing.

③ Thaw control DNA and template DNA, then place on ice.

④ Thaw the reaction solution premix in a 30˚C water bath (about 1 minute), and place on ice immediately after thaw.

- Before defrosting, check that the lid is not loose or the container is damaged.
- The indicated thawing time is a guideline, so please adjust accordingly.
- After thawing, place on ice and use immediately. If left for a long time, the performance will deteriorate.

⑤ Spin down the thawed reaction solution premix, control DNA, and template DNA in a tabletop centrifuge and place on ice

Protein Synthesis Reaction

Prepare dialysis solution:

① Add 2 mL of sterile distilled water to the dialysis solution premix, stir well, and leave at room temperature.

② Put two shaker balls into the dialysis solution.

③ Prepare the reaction solution. Mix the reaction solution mixture gently with a pipette until it becomes uniform, taking care not to create bubbles.

④ Add 50 µL of template DNA or control DNA, and 150 µL of distilled sterilized water into the reaction solution premix tube, and mix gently with a micropipette. Please be careful not to make bubbles.

Regents	Volume(µL)	Final conc.
Reaction solution premix	800	80% v/v
Template DNA [100(40~400) ng/µL]	50	5 (2~20) ng/µL
Sterilized water	150	–
Total	1000	

⑤ Gently transfer the reaction solution into the dialysis cup with a micropipette. Please be careful not to foam as much as possible.

⑥ Float the dialysis cup on the dialysis solution. Set the dialysis membrane so that air bubbles are not trapped under the

membrane, then close the lid.

⑦ Place the reaction device in a incubation shaker set at 30℃ and start shaking at a speed at which the upper shaker ball rotates slowly (Approximately 70 rpm at rotation radius of 25-30 mm).

⑧ Incubate for 6 hours with shaking.

*Shorter reactions (1-4 hours) may be suitable for the synthesis of labile proteins.

*Longer reactions (up to 16 h) may increase the amount of synthesis for some of durable proteins.

⑨ Remove the dialysis cup from the dialysis solution and collect the reaction solution with a micropipette.

⑩ Transfer the collected reaction solution into a 1.5 mL sample tube and place on ice for 5 minutes to stop the reaction.

⑪ If you need the total fraction including the insoluble fraction, use it as it is (total fraction). If the soluble fraction is required, centrifuge the reaction mixture at 15,000xg for 1 minute at 4°C and then use the supernatant (soluble fraction).

*Be sure to check the expression status by SDS-PAGE, etc., as some proteins may precipitate.

*If the reaction solution is left on ice for a long time after reaction completion, the expressed protein may precipitate or decompose. Use immediately or purify by an appropriate method.

*If the protein is to be purified by His-tag affinity resin, please dilute the collected reaction solution 5 to 10 times with equilibration buffer before apply on the affinity resin, otherwise the capture yield of the target protein onto the resin may decrease. The reaction solution contains moderate concentration of amines which interferes His-tag binding.

10. References

- Kigawa T. *et al.*, Cell-free Protein Synthesis Methods and Protocols (Spirin, A. S. & Swartz, J. R., eds.), 83-97, 2007
- Matsuda T. *et al.*, J. Biomol. NMR., 37 (3), 225-229, 2007
- Yabuki T. *et al*., J. Struct. Funct. Genomics, 8 (4), 173-191, 2007
- Seki E. *et al*., Anal. Biochem., 377, 156-161, 2008
- Yokoyama J. *et al.*, Anal. Biochem., 411, 223-229, 2011
- Matsuda T. *et al*., J. Bioorganic & Medicinal Chem., 20, 6579-6582, 2021

11. Troubleshooting

If the protein is not expressed or the expression yield is low, please refer to the FAQ on our website. If you have any other questions, please contact us.

Possible causes	Recommended measures
Reduced kit performance	(1) Please confirm that the product is within the expiration date indicated on the product label. (2) Please confirm that the product has been stored at an appropriate temperature. (3) If the product has been stored properly within the expiration date, please contact us.
Inappropriate template DNA	(1) Please check if the template DNA sequence is appropriate. (2) Please check if the concentration is appropriate by agarose gel electrophoresis. (3) When using plasmid DNA prepared with a commercially available plasmid purification kit, please perform additional purification by phenol/chloroform extraction followed by ethanol precipitation to remove a trace amount of residual nucleases. (4) Please try to confirm the expression using Musaibo Kun N100 or Start kit, that has a small scaled high-yield dialysis mode configuration. Some unstable proteins tend to degrade or precipitate during the protein synthesis reaction.

Problems due to the nature of the protein	(1) Lowering reaction temperature may reduce degradation or improve solubility although yield is decreased. Please try incubation at 25˚C. (2) When kits other than Quick or N mini are used, shortening reaction time may reduce degradation or improve solubility although yield is decreased. Please try 4 hours or less of reaction time. (3) Insertion of a tag sequence that improves solubility may change solubility of whole protein. The amount of expression varies greatly depending on the amino acid sequence especially at N-terminal region. (1) Please add or change N-terminal tag. (2) According to the protein specification, please try optimizing the construct by deletion, extension or mutation.

TAIYO NIPPON SANSO