

## Protein Synthesis Kit



# Membrane protein expression additive SetA

## Operating instructions

This product is used in combination with the following "Protein Synthesis Kit" sold separately. Do not use for any other purpose.

- Musaibo Kun SI (PEG-free)
- Musaibo Kun SI SS (PEG-free)
- Musaibo Kun N 100, Musaibo Kun N 1000
- Musaibo Kun N 100 SS, Musaibo Kun N 1000 SS

Prior to usage, we kindly request that you thoroughly peruse this manual to acquire a comprehensive understanding of the appropriate handling techniques.

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- In order to enhance the quality, the specifications, designs, text, etc. included in this manual are subject to change without prior notice.
- We make every effort to ensure the accuracy of all information provided in this manual. If you have any inquiries, comments, or notice any omissions, please contact us at the following email address.



Technical Information

## Safety Precautions

This product is intended for research purposes only. It is strictly prohibited to utilize this product, the protein, or any other components obtained from this kit for medical care and/or clinical diagnoses of humans and animals, or to incorporate them into beverages or food items.

This kit is designed for use by experienced individuals with expertise in general biochemical experiments and micropipette operations. Individuals lacking such experience must refrain from using this product.

Please be aware that we assume no responsibility for any issues that may arise from unauthorized use of this product, not in accordance with this manual.

## 1. Introduction

"Membrane protein expression additive" is a set of reagents for synthesizing membrane proteins using the "Protein Synthesis kit" sold separately. (The target product is listed on the cover page.) To ensure proper handling, we kindly request that you carefully read and familiarize yourself with the contents of this manual.

## 2. Features

Membrane proteins can be efficiently synthesized by simultaneously performing cell-free protein synthesis and forming proteoliposomes using surfactants and lipids.

We have obtained a license from RIKEN and have commercialized it. For details of this technology, please refer to the following reference.

## 3. Contents

- Additive M1: 67 mg/mL L- $\alpha$ -Phosphatidylcholine, 10% Digitonin 100  $\mu$ L
- Additive M2: 67 mg/mL L- $\alpha$ -Phosphatidylcholine, 10% Brij® S20 100  $\mu$ L

## 4. Storage

Store at -20°C or below.

Once opened, please use this product as soon as possible regardless of the expiry date to prevent oxidation of lipids.

## 5. Summary of Hazards and Risks



### 【GHS classification】

Acute toxicity (oral, dermal, inhalation) (Category 3)  
Harmful if swallowed (Category 4)

### 【Safety measures】

Wear protective gloves, protective clothing, protective glasses, and protective surfaces.

### 【First aid measures】

Should it get in contact with your skin and eyes, rinse with plenty of water. Please contact the poison control center or doctor if you are not feeling well.

### 【Disposal】

Dispose of contents and the container in an approved waste disposal site.

【Related Laws and Regulations】Not applicable.



## 6. Membrane protein Synthesis

### 6.1 Caution

Do not handle this product with bare hands.

Please ensure to wear appropriate safety goggles, gloves, lab coat, and other protective gear when handling this kit.

In the event that the solution of the kit comes into contact with the eyes and/or skin, it may cause inflammation.

- For detailed instructions on how to use the "Protein Synthesis Kit", please refer to the instruction manual attached to each kit.
- Since the conditions for synthesis may be different depending on the type of membrane protein, the micro dialysis method.
- **Point 1: Add this product only to the inner solution.**
- **Point 2: Since many membrane proteins tend to aggregate when boiled, it is recommended to prepare SDS-PAGE samples without boiling.**

### 6.2 Protein Synthesis Reaction

- Please perform the following steps 1) - 4) promptly.
- Please prepare the liquid volume according to the number of samples and reaction scale.

#### 1) Prepare the external solution (dialysis solution).

Prepare the external solution (dialysis solution) of the "Protein synthesis kit" according to the volume of the instruction manual for each kit.

**Do not add "membrane protein expression additive" to the external solution (dialysis solution).**

#### 2) Thaw and mix the "membrane protein expression additive".

If the solution does not dissolve at room temperature, warm it in a 30°C water bath for a few minutes, then mix by vortexing until a uniform suspension is achieved.

#### 3) Prepare the internal solution (reaction solution).

Add the "membrane protein expression additive" to achieve a final concentration of 10% v/v in the internal solution (reaction solution).

«Example of 100  $\mu$ L internal solution (reaction solution) Preparation»

#### 1.Musaibo kun SI (PEG-free)



| Reagents                                   | Volume ( $\mu$ L) |
|--------------------------------------------|-------------------|
| Internal solution                          | 77.5              |
| Unlabeled or SI-labeled amino acid mixture | 7.5               |
| Template DNA, Sterilized water             | 5.0               |
| "Membrane protein expression additive"     | 10.0              |
| Total                                      | 100.0             |

#### 2.Musaibo Kun SI SS (PEG-free)



| Reagents                                   | Volume ( $\mu$ L) |
|--------------------------------------------|-------------------|
| Internal solution                          | 68.5              |
| Unlabeled or SI-labeled amino acid mixture | 7.5               |
| Glutathione solution                       | 2.5               |
| Template DNA, Sterilized water             | 11.5              |
| "Membrane protein expression additive"     | 10.0              |
| Total                                      | 100.0             |

#### 3.Musaibo Kun N



| Reagents                               | Volume ( $\mu$ L) |
|----------------------------------------|-------------------|
| Reaction solution                      | 80.0              |
| Template DNA, Sterilized water         | 10.0              |
| "Membrane protein expression additive" | 10.0              |
| Total                                  | 100.0             |

4) Start the synthesis reaction according to the instruction manual.

## 7. References

- Shinoda, T. *et al.*, Sci. Rep., 6, 30442, 2016
- Shimono, K. *et al.*, Protein Sci., 18, 2160-2171, 2009
- EP Pat. No. 221374
- JP Pat. Nos. 5704677, 6722951

## 8. Stable Isotope-Labeled Amino Acid

Stable isotope-labeled amino acids for Musaibo kun are available for separate purchase. Please utilize them in combination with the kit.

| Product name                                                                 | Catalogue number |
|------------------------------------------------------------------------------|------------------|
| Amino acids mixture solution-UL-d 1 ml                                       | A107-0144        |
| Amino acids mixture solution-UL- <sup>15</sup> N 1 ml                        | A39-0072         |
| Amino acids mixture solution-UL- <sup>13</sup> C, <sup>15</sup> N 1 ml       | A40-0073         |
| Amino acids mixture solution-UL- <sup>15</sup> N, D 1 ml                     | A41-0074         |
| Amino acids mixture solution-UL- <sup>13</sup> C, <sup>15</sup> N, D 1 ml    | A42-0075         |
| Amino acids mixture solution-Lys, Arg- <sup>13</sup> C, <sup>15</sup> N 1 ml | A91-0128         |
| Amino acids mixture solution-Lys, Leu- <sup>13</sup> C, <sup>15</sup> N 1 ml | A92-0129         |
| Amino acids mixture solution-SeMet 1 ml                                      | A108-0145        |



### "Musaibo kun" Contact Information

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