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Kaneka Easy RNA Extraction Kit (for RT-PCR） Instruction Manual

Caution

- This product is for research purposes. Do not use for medical / clinical diagnosis of humans and animals. Also, do not use it as food, cosmetics, household items, etc.
- When using or disposing of this product, observe general precautions strictly in the laboratory, such as wearing protective equipment (protective gloves, protective goggles, etc.) and washing thoroughly with water if it gets in your eyes or if it gets on your skin. Please be careful about safety.

Main Features ／ Applicaitons

- This product will extract RNA for RT-PCR with a simple operation and in about 30 minutes.

Contents		
Solution A	400 µl	5
DNase I	100 µl	1

* This product is intended to be used as a template in RT-PCR.

How to use

Standard protocol

[For cultured cells]

1. **Transfer the cell culture medium to a centrifuge tube so that the number of cells is 103 to 106.**
2. **Centrifuge the tube at 1000 rpm for 3 minutes. Gently remove the medium components of the supernatant while not peeling off the collected cell pellets.**
3. **Add 100 µl of PBS (-) to the cell pellet and resuspend the cells by pipetting. Centrifuge the tube at 1000 rpm for 3 minutes, then remove the supernatant and collect the cells.**
4. **Add 20 µl of Reagent A to the centrifuge tube containing the cells and stir well and suspend by pipetting. Transfer the suspension to a new PCR tube.**
5. **Incubate the PCR tube containing the cell suspension in a heat block, or other devices, at 75 ° C for 5 minutes.**
6. **After cooling the PCR tube to room temperature, add 1 µl of DNase I and stir by pipetting. Then, incubate at 42 ° C for 10 minutes and at 75 ° C for 5 minutes in a**

heat block or other devices.

7. **Add the extract to the reaction system according to the protocol of the kit used. For example, extract [Note 1)] 1 to 2 µl is used for the reverse transcription reaction (in the case of a 25 µl reaction system). [Note 2)]**

[For Blood]

1. **Add 20 µl of Reagent A to the PCR tube.**
2. **Add 0.5 to 2.0 µl of fresh blood containing an anticoagulant such as heparin [Note 3)] to the above PCR tube and stir well by pipetting.**
3. **Incubate the above PCR tube in a heat block at 75 ° C for 5 minutes.**
4. **After cooling the PCR tube to room temperature, add 1 µl of DNase I and stir by pipetting. Then, incubate at 42 ° C for 10 minutes and at 75 ° C for 5 minutes in a heat block or the like.**
5. **5. Add the extract to the reaction system according to the protocol of the kit used. For example, extract Note 1) 1 to 2 µl is used for the reverse transcription reaction (in the case of a 25 µl reaction system). [Note 2)]**

Note 1 **Precipitates may be seen in the incubated solution. Removing the precipitate is not a required step, but to remove the precipitate, centrifuge the PCR tube at 4 ° C and 5000 rpm for 5 minutes to collect the supernatant.**

Note 2 **We recommend that you use the extract as soon as possible. When using the extract, keep it on ice, and when storing it, store it at -20 ° C or below.**

Note 3 **We have confirmed that RNA can be extracted from blood to which heparin and EDTA have been added.**

Precautions for Use

1. If the amplified fragment is not confirmed by RT-PCR using the standard protocol, it may be improved by performing the following operations.
   -Optimize the amount of sample used. (For cultured cells: range of 103 to 106 cells)
   -Perform work promptly without interrupting work in the middle of the protocol.
   -Use the extract promptly.
   -Optimize experimental conditions and primers.
2. RNA can be easily degraded by RNase. When working, pay close attention to contamination, and use RNase free products for all tubes and tips used. When using the extract, keep it on ice and store it at -20 ° C or below.

3. Depending on the cells used, there may be the effects of inhibitors in experiments using the extract as a template.

Storage / Expiration date

- Preservation method: This product will be delivered frozen. After receipt, store Reagent A and DNase I at -20 ° C or below. If Reagent A is frozen, thaw it completely before the experiment and stir well before use. Reagent A can be stored at -20 to 4 ° C after melting.
- Expiration Date: Stated on the packaging of this product.
- Please use immediately after opening this product.
- Repeated freezing and thawing may reduce the activity of DNase I. It may be possible to prevent a decrease in activity by dividing DNase I into small amounts in advance and using all of it each time.

Methods of Disposal

Be sure to wear protective equipment (protective gloves, protective goggles, etc.) when handling this product.

- Residual waste : If it is a small amount, absorb it with a paper towel or waste cloth and incinerate it.
- Contaminated containers and packaging : When disposing of empty containers and packaging after use, dispose of after completely removing the contents.

Warranty

- Please be noted that the scope of our responsibility is limited to the replacement of this product with a substitute if there is a problem with the product itself, and we will not be liable for any other damages, direct or indirect.

Contact

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