Repertoire Genesis, Inc.

Technical Guide

For sample processing and shipping

Ver.2016.03.31

Table of contents

※Please contact us if you have any questions about this material

Repertoire Genesis, Inc.
104 Saito Bioincubator, 7-7-15 Saito-Asagi
Ibaraki-shi, Osaka, 567-0085 Japan
TEL: +81-72-657-8686, FAX: +81-72-657-8687
E-mail: info@repertoire.co.jp

For sample processing and shipping

Repertoire Genesis, Inc. Ver.2016.03.31

1: Introduction

This material provides the protocol for processing the specimens and sending the samples to us for performing the TCR/BCR repertoire analysis. We conduct the repertoire analysis based on total RNA, which tends to be degraded easily. When total RNA is degraded, it is not possible to obtain a good result. Therefore, we conduct a quality check using Agilent 2200 TapeStation for all samples. Please be sure to read this document before you initiate sample processing. In this technical guide, we will introduce a stabilization example using RNAprotect cell Reagent or RNAlater and a shipping method.

2: Processing and shipping method

In our repertoire analysis, we use specimens that include the mRNA of T cells and B cells (blood, cultured cells, sorted cells and tissue).

The processing and delivery methods are shown in the following table. More details will be described separately as per the type of specimen, later in this document.

Sample Type	Blood (PBMC isolation)	Cultured cells sorted cells	Tissue	RNA*
Processing	RNAprotect cell reagent		RNAlater	
Shipping	Shipping with refrigerant pack			Shipping with dry ice

*We do not recommend you to send us RNA, because dry ice might be vaporized during transportation and delivery is sometimes delayed. If you want to send us RNA stored or freshly extracted in your lab, please call us.

3: PBMCs, cultured cells or sorted cells

After you isolate PBMCs from whole blood using density gradient centrifugation (e.g. Ficoll-Paque™, Lymphoprep™), you can stabilize RNA in cells and store the cells with RNAprotect cell reagent for up to 7days at 15-20℃ or for up to 4 weeks at 2-8℃. For culture cells and sorted cells, you can recover the cells by centrifugation and perform stabilization of cells with the RNAprotect cell reagent.

Reagents

RNAprotect® Cell Reagent (cat#: 76526, QIAGEN)

Stabilization protocol

■ **Cells in solution**

1. Wash cells (<1 × 10^7 cells) with medium or buffer (e.g, RPMI1640, Hanks, PBS)
2. Re-suspend cells with 1 vol. of medium or buffer
3. Add 5 vol. of RNAprotect cell reagent
4. Mix cells by shaking, pipetting, or vortexing

■ **Cell pellet**

1. Cell pellets (<1 × 10^7 cells)
2. 300 µL of RNAprotect cell reagent (>5 vol. of cell pellet)
3. Re-suspend the cells completely by vortexing.

Shipping protocol

1. Placed 1.5- or 2-mL sample tubes into a box with a lid
2. Put the sample box into the Styrofoam box according to our Packaging Instruction
3. Include a "shipping specimen list"
4. Wrap the package with strong tape (cloth duct tape)
5. Ship the package to address below by FEDEX
6. E-mail us the sample list and notify the tracking number

Shipping address:
Repertoire Genesis, Inc.
104 Saito Bioincubator, 7-7-15 Saito-Asagi
Ibaraki-shi, Osaka, 567-0085 Japan
TEL: +81-72-657-8686, E-mail: info@repertoire.co.jp

4: Tissue

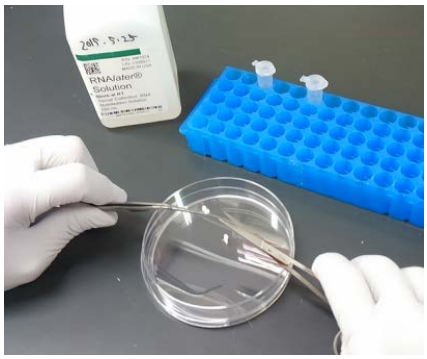
In case of fresh tissues, you can stabilize RNA by immersion of the tissues with RNAlater and store the immersed tissues for up to 7days at 15-20℃ or for up to 4 weeks at 2-8℃.

Reagents

RNAlater® Stabilization Solution (Cat#: AM7024, Ambion)

Stabilization protocol

1. Dispense 1 mL of RNAlater into 1.5-mL tubes in advance.
2. Trim the tissue specimens to a weight of 100 mg (approximately half the size of a soybean).
3. Cut into 3~5-mm^3 sections and immerse them in RNAlater.
4. After immersion, store the samples as soon as possible in the refrigerator (4℃) (overnight to 4 weeks).
5. For long-term storage, remove the RNAlater reagent after overnight immersion, and store the samples at −80℃ (optional)

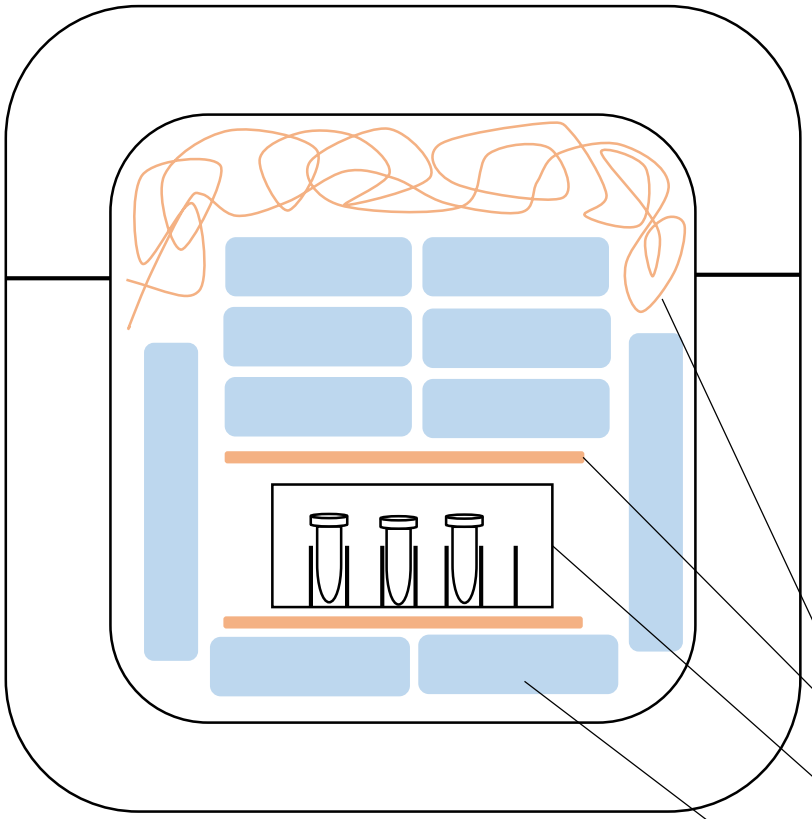


Shipping protocol

1. Placed 1.5- or 2-mL sample tubes into a box with a lid
2. Put the sample box into the Styrofoam box according to our Packaging Instruction
3. Include a "shipping specimen list"
4. Wrap the package with strong tape (cloth duct tape)
5. Ship the package to address below by FEDEX
6. E-mail us the sample list and notify the tracking number

Shipping address:
Repertoire Genesis, Inc.
104 Saito Bioincubator, 7-7-15 Saito-Asagi
Ibaraki-shi, Osaka, 567-0085 Japan
TEL: +81-72-657-8686, E-mail: info@repertoire.co.jp

Packing Instruction



Thick-wall Styrofoam ice box
(Ex. 15" x 15" x 12")

Required ice pack: 10 kg (3-days)

- Freeze ice packs overnight
- Seal sample tubes (1.5 mL Eppendorf tube) and place them in a sample box.
- Use a Styrofoam box with walls 2 inches thick or more
- Place a ice pack in the bottom of box
- Place a thin layer of 3-4 paper towels (for insulation)
- Put the sample box on it
- Fill the box with necessary ice packs
- Fill all gaps along box and between ice pack and box with paper towel
- Seal the box with Sealing Tape
- Ship the box by the FEDEX

Paper towel

Sample box

Ice pack