Ver. 1911-00

PureHelix™ *Total RNA* Purification Kit

[Virus, Animals, Plants, Bacteria] (Column Type)

Kit contents

PureHelix™ *Total RNA* Purification Kit			
Cat. No.	**RPT50** **(50 preps/kit)**	**RPT100** **(100 preps/kit)**	**RPT200** **(200 preps/kit)**
Column set (with cap) 50ea/**Blue Box**	1 box	2 box	4 box
Buffer RCLB	30 ml	60 ml	120 ml
Buffer RWB1	32 ml **(Add 8 ml ethanol)**	64 ml **(Add 16 ml ethanol)**	128 ml **(Add 32 ml ethanol)**
Buffer RWB2	8 ml **(Add 32 ml ethanol)**	16 ml **(Add 64 ml ethanol)**	32 ml **(Add 128 ml ethanol)**
MaxBinder™ solution	5 ml	10 ml	20 ml
EB (RNase-free)	5 ml	10 ml	10 ml x 2ea
Instruction for use	1 ea	1 ea	1 ea

Description

PureHelix™ *Total RNA* Purification Kit [Virus, Animals, Plants, Bacteria] is designed for rapid, pure, and high yield isolation of total RNA from small amounts of various samples including blood, animal and plant tissues, bacteria and virus. This kit is suitable to the rapid preparation of nucleic acids for molecular diagnostics using conventional and real-time RT-PCR technologies. Due to elimination of phenol, handling of the kit is safe and no harmful waste is produced. The purified total RNA can be used in a number of downstream applications.

Applications

Preparation of total RNA for RT-PCR or quantitative RT-PCR

Preparation of nucleic acid sample for molecular diagnostics

Store

Ambient temperature

Quality control assay data

Functional analysis

PureHelix™ *Total RNA* Purification Kit [Virus, Animal, Plant, Bacteria] was tested for the isolation of total RNA from blood, animal tissue, plant leaf tissue and bacterial cell.

Quality authorized by : Yountaek Go

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Protocol

Important things to do before starting

- ***β*-Mercaptoethanol** (not provided in this kit) must be added to Buffer RCLB before use. **Add 10 µl** of ***β*-Mercaptoethanol per 1 ml** of **Buffer RCLB.** The *β*-Mercaptoethanol(2-ME) containing Buffer RCLB is stable for 1 week at room temperature.
- Prepare **100% Isopropanol** (not provided in this kit.)
- Prepare **70% Ethanol for Plant total RNA extraction** (not provided in this kit.)
- Before using for the first time, **add absolute ethanol into the Buffer RWB1** as indicated on the bottle to obtain a working solution.
- Before using for the first time, **add 4 volumes of absolute ethanol into the Buffer RWB2** to obtain a working solution. **※ If you need more Buffer RWB2, you may use 80% ethanol (RNase-free).**

1. Sample Preparation and Cell Lysis.

Animal Tissue

1) **Add 300 µl of Buffer RCLB (2-ME added) to 20 ~ 50 mg fresh tissue sample** in a microcentrifuge tube and homogenize using an appropriate apparatus, such as hand-operated pellet pestle or motor-driven grinder.
2) **Add additional 200 µl of Buffer RCLB (2-ME added)** to the homogenized sample and vortex for 15 ~ 30 seconds.
   ※ Sample volume should not exceed 10% volume of Buffer RCLB.
3) Centrifuge at 12,000 rpm for 10 min and transfer the supernatant into a microcentrifuge tube.
4) **[Optional]** In case that debris still remains in the supernatant, add 500 µl chloroform and vortex for 15 ~ 30 seconds. Centrifuge at 12,000 rpm for 10 min and transfer the upper aqueous phase to a microcentrifuge tube.

Plant Tissue

1) **Add 350 µl of Buffer RCLB (2-ME added) to 20 ~ 100 mg fresh tissue sample** in a microcentrifuge tube and vortex for 15 ~ 30 seconds.
2) Centrifuge at 12,000 rpm for 10 min and transfer the supernatant into a microcentrifuge tube.
3) **[Optional]** In case that debris still remains in the supernatant, add 350 µl chloroform and vortex for 15 ~ 30 seconds. Centrifuge at 12,000 rpm for 10 min and transfer the upper aqueous phase to a microcentrifuge tube. **※ Chloroform is not provided.**

Blood

1) Transfer **100 µl of non-coagulating blood** to a microcentrifuge tube.
2) **Add 500 µl of Buffer RCLB (2-ME added)** and vortex for 10 seconds.

Cells from Nasal or Throat Swabs

1) Add **500 µl of Buffer RCLB (2-ME added)** to a microcentrifuge tube.
2) Brush a sterile, single-use cotton swab or Buccal Swab Brush inside the nose or mouth of the subject.
3) Cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the Buffer RCLB (2-ME added). Close the tube. Vortex and incubate at room temperature for 5 min.

Cells grown in monolayer

1) Put off culture media.
2) Add **500 µl of Buffer RCLB (2-ME added) per 1 ~ 5 x 10^6 cell.**
3) Lyse cells and homogenize the sample by passing through a pipette several times.

Cells grown in suspension

1) Pellet **1 ~ 5 x 10^6 animal, plant, or yeast cells, or 1 x 10^7 bacterial cells**.
   Occasionally, enzymatic lysis or mechanical disruption is required for the cell-wall disruption of some yeast and bacterial cells.
2) Discard the supernatant and then add **500 µl of Buffer RCLB (2-ME added).**
3) Lyse the sample by repetitive pipetting or vortexing for 10 seconds.

2. Column Activation [Optional]

1) Place a Spin Column into a 2 ml collection tube.
2) Add **100 µl of MaxBinder™ solution** into the Spin Column.
3) Centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.
   ※ These steps are required for the best yield.

3. Column Loading

1) Add **200 µl** of **Isopropanol** to the prepared cell lysate and vortex.
   ※ Plant tissue: Add 350 µl of 70% ethanol (not Isopropanol) to the prepared cell lysate and vortex.
2) Load **700ul** of **mixture** directly into a spin column sitting in a 2 ml collection tube and centrifuge at **12,000 rpm for 1 min**. Discard the flow-through.

4. [Optional] DNase I treatment : On-column protocol

※ RNase-free DNase I can be applied with on-column DNase digestion for the elimination of DNA.

※ DNase I is not provided in this kit. We recommend to use HelixZyme™ RNase-free DNase I (Cat No. RDN1500) for this step.

1) Add **350 µl** of **RWB1** (ethanol added) into the spin column, and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.

2) Add **2.3ul (27.3 Kunitz unit)** of **RNase-free DNase I** to **77.7ul** of **DNase reaction buffer**. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from side of the tube.
[Caution] DNase I is highly sensitive to a physical damage and do not vortex or vigorous pipetting in this step.
3) Add **80ul** of the **DNase mixture** into the spin column and incubate at **Room temperature (20~30℃)** for **15min**.
4) Add **350 µl** of **RWB1** (ethanol added) into the spin column, and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through

5. Column Washing

1) Add **700 µl** of **RWB2** (ethanol added) or 80% ethanol (RNase-free) into the spin-column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
2) Centrifuge again at 12,000 rpm for 2 min to remove residual ethanol.

6. Elution of RNA

1) Place the spin column into an RNase-free microcentrifuge tube.
2) Add **40 ~ 50 µl** of **EB** to the center of the column membrane, and incubate at room temperature for 1 min.
3) Centrifuge at 12,000 rpm for 1 min, and store RNA at -20 or -70℃.

[Appendix] DNA digestion of RNA: Clean-up Protocol

To remove DNA from RNA samples, this DNase digestion and clean-up protocol could be applied.

1) Add **1µl (12 Kunitz units) RNase-free DNase I (Cat No. RDN1500)** and add **5µl** of **10x Dnase reaction buffer** to the prepared **RNA solution (50µl)** and mix gently by inverting.
2) Incubate at **Room temperature(20~30℃)** for **15min**.
3) Add **350µl** of **RCLB** and **150µl** of **isopropanol** and mix by pipetting.
4) Transfer the mixture directly into the spin column sitting in a 2 ml collection tube and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
5) Add **700 µl** of **RWB1** (ethanol added) into the spin column, and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
6) Add **700 µl** of **RWB2** (ethanol added) or **80% ethanol** (RNase-free) into the spin-column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
7) Centrifuge again at 12,000 rpm for 2 min to remove residual ethanol.
8) Place the spin-column to an RNase-free microcentrifuge tube and add **40 ~ 50 µl** of **EB** into the spin-column. Incubate at room temperature for 1 min.
9) Centrifuge at 12,000 rpm for 1 min and store at -20 or -70℃.

Products

Cat. No.	Products	Size
RPT50	PureHelix™ Total RNA Purification Kit [Virus, Animal, Plant, Bacteria] (Column type)	50 preps
RPT100	PureHelix™ Total RNA Purification Kit [Virus, Animal, Plant, Bacteria] (Column type)	100 preps
RPT200	PureHelix™ Total RNA Purification Kit [Virus, Animal, Plant, Bacteria] (Column type)	200 preps
RDN1500	HelixZyme™ RNase-free DNase I	1500KU

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