AnaPrep Total RNA Extraction Kit

Cat. No. Z1322015 Process Time: 50-55 minutes

Intended Use

AnaPrep Total RNA Extraction Kit is used with the AnaPrep 12 instrument for extraction of total RNA from whole blood, blood cells, animal tissue, plant tissue, yeast or cultured cells

Application

Total RNA extracted from AnaPrep Total RNA Extraction kit can be used in a number of downstream application including: RT-PCR, qPCR, sequencing (NGS), Microarray, and Northern blot.

Number Of Tests

36 and 48 extractions

Kit Components

Kit Contents	Quantity
	ZP02015-48
Reagent Cartridge	48 pcs (4x6x2)
Reaction Chamber	48 pcs (4x6x2)
Tip Holder	48 pcs (6X4x2)
Filter Tip	50 pcs
Piercing Pin	50 pcs
Sample Tube (2 mL)	50 pcs
Elute Tube (1.5 mL)	50 pcs
RL A Buffer	25ml
RL B Buffer	25ml
Filter column	50 pcs
Collection tube	50 pcs
Barcode Paper	1 pc

Reagent Cartridge Content

well-1	Proteinase K solution	40 µl
well-2	Lysis Buffer 1	720 µl
well-3	Binding Buffer 1	1300 µl
well-4	Magnetic Bead Solution	800 µl
well-5	Washing Buffer 1	1000 µl
well-6	Washing Buffer 2	1000 µl
well-7	Washing Buffer 3	1000 µl
well-8	RNase-free water	1000 µl
well-9	Rnase-free water	1000 µl

Storage

- AnaPrep Total RNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The kits are stable for 15 months under the proper conditions.
- After extraction, store RNA at -60 to -80℃ immediately, repeated freeze–thawing is not recommended. Always handle RNA on ice for downstream analysis.

Starting Material

Sample Type	Sample Volume (Amount of starting material)	Elution Volume
Whole blood	200-400 µl* (WBCs no. about 10^6)	50-200µl***
PBMCs	Up to 50 µl (suspended in 200µl with RL buffer)	
Tissue	10-40mg (Lysed and suspend with RL buffer)**	
Cultured cells	200-400 µl suspension of primary or cultured cells (cell no. < 5 x 10^6)	
Plant tissue	Up to 100mg	
Yeast	Up to 100mg	
Controls/internal control	Add controls/internal control in the extraction procedure to facilitate final downstream analysis.	

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. Using excess quantity is not helpful in total RNA extraction.

* Blood cells require RBC lysis procedure before extraction

**Animal tissue, plant tissue, and yeast require homogenization before extraction

*** After extraction, store RNA at -60 to -80°C immediately, repeated freeze–thawing is not recommended

Reagents to be supplied by user

Reagent	Description	Preparation
β -mercaptoethanol (β -ME)	β -ME reduces disulfide bonds and denatures the RNase thus reduces RNase released during cell lysis	Add 10 µl β-ME per 1 ml RL lysis Buffers*. It can be stored at RT for up to one month.
Red blood cells lysis buffer (RBC lysis buffer)	Lyse erythrocyte from whole blood	10xRBC lysis buffer(100ml) 8.29g NH_4Cl (1.5M) 1g $KHCO_3$ (100mM) 0.0372g Na_2EDTA (10mM) Adjust pH7.2-7.4 with HCl.

		Then 0.2 mm filtered. Stable for 6 months at 4℃. Dilute fresh to 1x before use.
DNase	To eliminate DNA contamination	Novagen RNase-free DNaseI (69182-3CN)

*RL lysis buffers means RLA and RLB Buffers. Dispense the β-ME in a fume hood and wear appropriate protective clothing

Before starting

- β-Mercaptoethanol (β-ME) must be added to RLA or RLB buffer before use.
- Homogenization is necessary for animal tissue, plant tissue and yeast before extraction. Add RLA or RLB Buffers to samples for homogenization
- Minimization of RNase contamination is critical throughout the entire process. Always wear clean glove and use RNase-free filter tip. Use RNAse decontaminants (e.g. RNaseZap) to clean work area
- RNA stabilizing reagent (e.g. RNAlater) is an option to protect the RNA if the samples cannot be processed in a RNase-free work area

Sample preparation

Sample	Procedure
Whole blood	**1. Use fresh whole blood sample for isolation. For optimal results, blood samples should be processed within a few hours of collection and keep in 4℃. Freezing blood is not recommended.** **2. The blood sample should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used.** **3. If using whole blood samples which have extreme high WBCs no. (more than 10000/μl) or concentrated PBMCs(peripheral blood mononucleated cells), decrease the input volume for extraction is recommended (total WBC number less than 5 x 10^6).**

	4. Fresh prepare 1x RBC lysis buffer 5. Add ice-cold two volume of RBC lysis buffer to one volume of blood sample 6. Invert 3-5 times, incubate on ice for 10-15 min 7. Centrifuge at 1000 x g, 10min, 4℃ 8. Remove supernatant 9. Resuspend pellet with 220 μl RLA Buffer 10. Transfer 200 μl to sample tube for extraction
PBMCs (Peripheral Blood Mononucleated Cells)	1. Resuspend PBMCs with 220μl RLA Buffer 2. Vortex for 10 sec 3. Transfer 200 μl to sample tube for extraction
Tissue	1. To prevent degradation by intracellular RNase, it is important that tissues are either flash-frozen in liquid nitrogen or stored at –70°C, or processed immediately following excision. 1. Use RNA stabilized reagent (e.g. RNA later) to treat tissue to protect the RNA if sample cannot be frozen immediately. Frozen tissue should not be allowed to thaw during handling (e.g., weighing, cutting, homogenizing). Keep sample on ice. 2. Add 220μl RLA Buffer to tissue; make sure the sample if completely immersed in buffer. Increase RLA buffer input amount up to 440 μl if tissue sample is large. 3. Homogenized tissue with homogenizer 4. Quickly spin down the homogenate with a counter top centrifuge for <5 sec 5. Remove all the homogenate to the filter column (supplied in the kit) sitting in the collection tube 6. Centrifuge at 1000 x g, 5min, 4℃ 7. Transfer 200-400μl to sample tube for extraction
Cultured cells	【Protocol 1】Suspension culture 1. Harvest cell 2. Centrifuge at 1000xg, 5min, 4℃ 3. Remove supernatant completely 4. Resuspend cell pellet with 22oml RLA Buffer 5. Vortex for 10 sec

	6. Transfer 200μl to sample tube for extraction **【Protocol 2-1】Monolayer culture** **1. Trypsinize the cells** **2. Harvest the cell in PBS** **3. centrifuge at 300xg, 5min, 4℃** **4. Remove supernatant** **5. Resuspend pellet with 220 μl RLA Buffer** **6. Vortex for 10sec** **7. Transfter 200μl to sample tube for extraction** **【Protocol 2-2】Monolayer culture** **1. Scrape the cells with 220-440μl RLA Buffer** **2. Vortex for 10sec** **3. Transfer 200-400μl to sample tube for extraction**
Plant tissue/ Yeast	**1. Up to 100 mg of sample is first ground in liquid nitrogen or frozen** **2. Add 220 -440μl RLA or RLB Buffer to sample, make sure the sample is completely immersed in buffer. Most plant cells use RLA Buffer for disruption and denaturing sample. However, some tissues, such as milky endosperm of maize or mycelia of filamentous fungi, solidify in RLA Buffer, making the extraction of RNA impossible. In these cases, RLB Buffer should be used instead.** **3. Homogenized tissue by homogenizer** **4. Remove lysate to filter column (supplied in the kit) sitting in collection tube** **5. Centrifuge at 1000 x g , 5min, 4℃** **6. Transfer 200-400μl to sample tube for extraction**

Purification Protocol

Protocol name	Sample vol. Elute vol.	Description
Total RNA	100-400µl 50-200 µl	Extraction Total RNA and DNA from sample

Controls/ internal control

Using appropriate controls for downstream analysis:

Type	Description	Location
Positive control	Use sample positive for target	Place in sample tube
Negative control	Use sample negative for target	Place in sample tube
Internal control(IC)	Use a defined quantity for control	Place in the round well of the reaction chamber

Quality Control

In accordance with BIOCHAIN'S ISO-certified Quality Management System, each lot of AnaPrep Total RNA Extraction Kit is tested against predetermined specifications to ensure consistent product quality.