RNApure High-purity Total RNA Fast Extraction Kit

For fast extraction total RNA from cells/tissue of animals/plants

Contents, storage, stability

Contents	**Storage**	**RP1201** **20 preps**	**RP1202** **50 preps**
Buffer RL	4℃ in dark	25 ml	55 ml
Buffer RE	RT	15ml	30ml
Buffer RW	4℃(1 month) -20℃(long-term)	6 ml	15 ml
		Add ethanol before use	
RNase-free H_2O	RT	5 ml	10ml
70% Ethanol	RT	4ml RNase-free H_2O	9ml RNase-free H_2O
		Add ethanol before use	
Dnase (only for typeII)	-20℃	40ul	100ul
10×Buffer (only for typeII)	-20℃	60ul	150ul
RNase-free Binding column RA	RT	20 pcs	50 pcs
Collection Tube(2ml)	RT	20 pcs	50 pcs

Store at room temperature for up to 12 months.

Notices

1.Add ethanol to Buffer RW and 70% ethanol bottle before first use, make a mark to avoid add again.

2.All the solutions are clear, precipitations may form upon low temprature, water bath for a few minutes before use.

3. Reagents stored at low temperature(4℃ or -20℃) may form precipitation,which will influence the usage, thus tranport and store the reagents at room temprature(15℃-25℃), Transport Buffer RL at room temprature, store at 4℃ in dark upon receiving.
4. Avoid the reagents open in air, it will cause evaporation, oxydation, changing of PH, etc. Cover the reagents tightly after each use.

Principle

Modified Guanidine thiocyanate/phenol lysate cells and inactivate RNase in one-step, then total RNA is selectively bound to the silica membrane in high ionic salt condition. Contaminants such as celluar residuals, proteins,etc are removed through a series of washing-centrifuge steps, at last high-purity of total RNAs are eluted in RNase-free water from silica membrane.

Features

1. The silica membranes in the Binding column are special membranes from well-known company. The difference of binding capacity between columns is very small and the repeatability is good.
2. Stable Guanidine thiocyanate/phenol method, no need of isopropanol precipitation and ethanol washing, RNA eluted directly from binding column, avoid the disadvantages of excess dry or hard to elute.
3. Unique Buffer RL solution, remove the genome DNA contamination effectively.
4.The purity of extracted RNA is higher after multiple times of protein washing steps and removal of useless 5s RNA.

Important notes(Read this before experiments)

1.In order to avoid RNA degrade, all the steps should be handled at 4℃. Speed of Cenutrifuge should be capable of 13，000 rpm.

2. Buffer RL and Buffer RE contains irritation chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Washing immediately using lots of water or saline if contact with reagents.

3.In case of RNase contamination, follow the following rules while handling.

*Skin always carry bacteria and mould, they may be the source of RNase. Wear dissposible gloves during the whole procedure.

*Use sterized, dissposible plastic apparatus and tips, avoid cross contamination by using public instruments or consumables.

*Use RNase-free glass or plastic consumables. Bake the glass consumables in lab oven at 150℃ for 4 hours. Bath the plastic consumables in 0.5 M NaOH for 10 minutes, wash and autoclave for use.

4. Consider of the environmental protection, there is no chloroform in this kit, users need to prepare chloroform by themselves.

5. After eletrophoresis, high quality of RNA products show two obvious rRNA bands, which are ~ 5Kb (28S) and ~ 2Kb (18S). The brightness is about 2:1. Sometimes there are also ~0.1kb and 0.3Kb (5S, tRNA) bands too.

Sometimes 4 or 5 bands of special plant tissues from different species is also normal, such as discontinuous high molecular bands between 7Kb and 15Kb from immature precursors of RNA, inhomogeneous nuclear RNA or small nuclear RNA.

6.Test OD_{260}/OD_{280} after RNA eluted in Buffer TE, the OD_{260}/OD_{280} may decrease if elute RNA with water, because OD_{280} may increase because of the low value of ionic strength and PH.

7.Before adding chloroform and after homoginate in Buffer RL, samples can be stored at –60℃-70℃ for more than 1 month.

Protocol

Add ethanol to Buffer RW and 70% ethanol before first use.

1. Homogenate

a. Tissues Homogenate tissue samples using glass or Homogenizer, ground the tissues stored in liquid nitrogen with mortar and pestle, add 1 ml Buffer RL per 50~100mg tissues and then homogenate. Normaly the volume of tissue should not exceeds 10% the volume of Buffer RL.

b. The monolayer cells Add 1ml Buffer RL to the 3.5cm culture plate to lysis the cells. pippet to mix well. The amount of RL required for lysis (1 ml per 10 cm

^{2}) was determined by the area of the culture plate rather than by the number of cells.Commonly, add Buffer RL 1ml to cell culture bottle, shake gently to mix the buffer and cells, and inactivate the RNase, pippet gently to mix. Inadequate Buffer RL will cause DNA contamination in the extracted RNA.

c.Suspended cells Centrifuge to precipitate the cells. Mix lysed cells in Buffer RL repeatedly by pipetter. Add 1ml Buffer RL per 5~10 x 10^6 animal cell, plant or yeast cell or each 1 x 10^7 bacteria. Avoid washing cells before adding Buffer RL, which will increase the possibility of mRNA degradation. It may need homogenizer to lysis certain yeasts and bacteria.

2.Vortext the homogenated sample for at least 1 minute and incubated at 15-30℃ for 5 minutes to completely decompose the ribosome.

3. **Optional step** Centrifuge at 12,000 rpm at 4 ℃ for 10 minutes. Transfer the supernatant to a new RNase-free tube.

Optional steps is needed if the samples are rich of protein, fat, polysaccharide or extracellular substances, such as musle, fat tissue or the tuber part of a plant.

Centrifuge 12,000rpm at 2~8℃ for 10 min, remove undisolved substances after homogenate, the precipitation contains cellular outer membrane, polysaccharide and DNA, and upper layer phase contains RNA.

4. Add 0.2ml chloroform per 1ml Buffer RL. Tightly cover the sample, vortex for 15s and incubate at room temperature for 3 minutes.

5.The sample will be divided into three layers after centrifuge 12,000 rpm at 4℃ for 10 minutes: the lower organic layer, the middle layer and upper colorless aqueous layer, RNA stay in the aqueous layer. The volume of the aqueous layer is about 60% of the Buffer RL volume. Transfer the water layer to a new tube for next step.

6. Add 1 volume of 70％ ethanol(Check whether ethanol is added), mix by upside down(precipitation may form). Transfer the liquid with precipitation together to Binding column RA(placed on the collection tube).

7. 10,000rpm for 45s, discard the liquid in the tube, place the Binding column RA back to the collecting tube.

8. Add Buffer 500ul RW(check whether ethanol is added), 12,000 rpm for 60s, discard the liquid in the tube;
9. Place the Binding column RA back to the collecting tube, remove the washing buffer utmost, in case the remaining ethanol inhibit the enzyme digestion.
10. **Optional step** Add 30μl Digestion Buffer in the middle of the sillica membrane, incubate at 37℃ for 15-30 minutes. The content of Digestion Buffer: RNase-Free DNase 2ul， DNase 10×Reaction Buffer 3ul， RNase-Free Water 25ul.

    The amount of RNase-Free DNase is based on DNA amount, 1μl RNase-Free DNase could digest 1μg RNA, the amount of 10×Reaction Buffer is based on total volume.
11. Add 500μl Buffer RE, place at room temprature for 2 minutes, 12,000rpm for 45s, discard the collected liquid.
12. Add 500μl Buffer RW(check whether ethanol is added), 12,000 rpm for 60s, discard the liquid in the tube.
13. Repeat step 12.
14. Place the binding RA column back to collection tube, 12,000 rpm for 2 min, remove washing buffer utmost, in case of remaining ethanol in the washing buffer inhibit downstream reactions.
15. Place the Binding column RA on a new RNase-free Centrifuge tube, add 50-80μl(elution volume based on predicted yield of RNA) RNase-free water(preheated in 65-70℃ water bath will be better), place at room temprature for 2 min, 12,000 rpm for 1 min.

    Transfer the eluted liquid in the tube back to the Binding column or add another 30μl RNase-free water if more yield of RNA is needed, 12,000 rpm for 1 min, mix the eluted liquid.

Trouble shooting

Problems	Possible causes	Solutions

Low RNA yield	Incomplete lysate or homogenate.	Grind completely in liquid nitrogen, shake violently or mix with pipettor to help lysate. Homogenate could increase production. Grind fresh tissue or plant tissue with no liquid nitrogen, grind directly with Buffer RL.
	Samples or lysate kept at -20℃ or -70℃ for too long	Long term storage may decrease the production of RNA, samples or lysate should be handled as soon as possible
	RNA content in tissue is low	RNA content from different types of tissue or cells are different, increase sample amount if the RNA content is low.
	Exceed the binding capacity of Binding column	Use more Binding column for one sample, then mix the RNA product.
	Forget to add ethanol to Buffer RW and 70% ethanol	**Add proper ethanol to Buffer RW and 70% ethanol before the first use**

OD_{260}/OD_{280} <1.6	RNA eluted in water not Buffer TE, OD_{280} will increase when in low ionic concentration or low PH, leads to low OD_{260}/OD_{280} value.	Use Buffer TE to elute the RNA when testing.
	Contaminated with protein or phenol	Don't mix the central phase and lower phase in step 5, don't forget step 8.
Contaminated with DNA	Sample amount exceeds the capacity of Buffer RL	Decrease the sample amount
	Organic solvent in the samples(such ethanol, DMSO,etc),strong buffer or alkaline solutions.	Avoid substances might change the characteristics or PH of Buffer RL

	Contaminated with middle payer liquid during transfer the supernatant.	Don’t pippet any liquid from middle phase during transfer the supernatant in step 5.
RNA degrade, poor integrity	RNase is not inactivated on consumables and reagents.	Prepare consumables and reagents according to the notes.
	Didn’t treat or freeze samples imidiately after the samples are taken out, RNA degrade before extraction.	Treat samples as soon as possible, or store in liquid nitrogen or -70℃.
	Extracted RNA not stored in -20℃ or -70℃.	Stored in liquid nitrogen or -70℃
	RNA degrade during the extraction process.	Make extraction as fast as possible, centrifuge in low temprauture, handle RNA on ice.

Downstream RT-PCR failure	Forget to do step 11, or the binding column touched the liquid in the collection tube while handling, caused eluted RNA contaminated with ethanol, which inhibit the reverse transcription.	Make sure to do step 11, take the Binding column carefully, or dry in the air, let the ethanol evaporate.