Fast Viral Total RNA Extraction Kit

Contents, Storage and Stability

Contents	Storage	RP9011 50 preps	RP9012 100 preps
Buffer RLS	4℃ in dark	55 ml	55 ml×2
Carrier	4℃ -20℃ for long	310 ul	310 ul×2
Buffer RE	Room Temprature	30ml	60ml
Buffer RW	4℃(1 month) -20℃(long term)	15 ml	25 ml
		Add proper ethanol before use	
RNase-free H_2O	Room Temprature	10ml	20ml
70% Ethanol	Room Temprature	9ml RNase-free H_2O	18ml RNase-free H_2O
		Add proper ethanol before use	
RNase-free Column RA	Room Temprature	50 pcs	100 pcs
Collection Tube(2ml）	Room Temprature	50 pcs	100 pcs

It won't affect it's using effects within 12 months if stored in room temprature.

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 shoud be 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℃)

4. Avoid the reagents open in air, it will cause evaporation, oxydation, changing of PH, etc. Cover the reagents tightly after each use.

Principle

Erythrocytes and leukocytes are lysed and RNases are inactivated in one-step, RNA is selectively bound to the silica membrane in high ionic salt condition. Contaminants such as celluar residuals, proteins,etc are washed away through a series of washing-centrifuge steps, at last high-pure of total RNAs are eluted in RNase-free water from silica membrane.

Features

1. The silica membranes in the absorbing column are special adsorption membranes from well-known brand in the world. The difference of adsorption capacity between columns is very small and the repeatability is good.

2. Stable Guanidine thiocyanate/phenol reagent, no need of Isopropanol precipitation and ethanol ringing, RNA eluted directly from spin column, avoid the excess dry and hard to dissolve.

3. Unique Buffer RLS 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℃. Centrifuge speed more than 13，000 rpm.

2. **Buffer RLS 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. DNase I can be used to digest DNA through columns.

4.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, glass consumbles Bake the glass consumables in lab oven at 150°C for 4 hours. Bath the plastic consumables in 0.5 M NaOH for 10 minutes, wash and autoclave for use.

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

6. 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.

7.Test OD260/OD280 after RNA eluted in Buffer TE, the OD260/OD280 may decrease if elute RNA with water, because OD280 may increase because of the low value of ionic strength and PH.

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

Protocol

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

Add RNase-free H2O to Carrier before first use.

1. Homogenate

a. Biofluid

Add 0.75ml Buffer RLS and 6ul carrier to each 0.25ml liquid samples(Serum, Plasma and cerebrospinal fluid,etc), pippet to mix the samples. At least 0.75ml Buffer RLS for each 5~10×10^6 cells. The ratio of Buffer RLS and Liquid Sample is 3:1.

b. Tissues

Homogenate tissue samples using glass or Homogenizer, add 0.75ml Buffer RLS

and 4uL Carrier to each 50~100mg tissues or 0.25ml tissue suspension. Normaly the volume of 50~100mg tissue is less than 0.25ml, add sterized water to adjust the volumn to 0.25ml, ensure the ratio is 3:1.

c. The monolayer cells

Add 0.3ml-0.4ml Buffer RLS and 4uL Carrier to the 3.5cm culture plate to lysate the cells. pippet to mix well. The amount of RLS required for lysate (0.3-0.4 ml per 10 cm 2) was determined by the area of the culture plate rather than by the number of cells. There is no need to add water to the culture plate, because the remaining culture liquid already diluted the Buffer RLS.

d. Suspended cells

Centrifuge to precipitate the cells. mix cells were lysed in Buffer RLS repeatedly by pipetter. Add 0.75ml Buffer RLS and 4uL Carrier to each 5~10 x 10^6 animal cell, plant or yeast cell or each 1 x 10^7 bacteria. Same as step B, the volume of the sample is adjusted to 0.25 milliliters with sterilized water. Avoid washing cells before adding Buffer RLS, which will increase the possibility of mRNA degradation. It may need homogenizer to lysate certain yeasts and bacteria.

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

3. **Optional step:** If there is an obvious insoluble mass in the solution, centrifuge at 12,000 rpm at 4 C for 10 minutes. Transfer the supernatant to a new RNase-free tube.

4. Add 0.2ml chloroform to each 1ml Buffer RLS. 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°C 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 RLS volume. Transfer the water layer to a new tube for next step.

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

7. 10,000rpm for 45s, discard the liquid in the tube, put the absorbing column back to the collecting tube.

8. Add Buffer 500uL Buffer RE, 12,000 rpm for 45s, discard the flow through.

9. Add 700uL Buffer RW(check wether ethanol is added), 12,000 rpm for 60s, discard the flow through.

10. Add 500uL Buffer RW, 12,000 rpm for 60s, discard the flow through.

11. Place the absorbing 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.

12. Place the Absorbing column RA to a new RNase-free Spin column, add 20-30μl 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 absorbing column or add another 30μl RNase-free water if more RNA is needed, 12,000 rpm for 1 min.

The larger the elution volume is, the higher the elution efficiency is. Reduce the elution volume to get higher concentration of RNA, but the minimum volume should be no less than 30ul. Small volume will reduce the elution efficiency of RNA thus reduce the yield of RNA.

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 RLS.
	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 adsorbing capacity of adsorbing column	Use more adsorbing column for one sample, then mix the RNA product.
	Forget to add ethanol in Buffer RW and Buffer RW	**Add proper ethanol to Buffer RW and 70% ethanol at the first use**
OD_{260}/OD_{280} <1.6	RNA eluted in water not Buffer TE, OD280 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 integraty	RNase is not inactivated on consumables and reagents.	Prepare consumables and reagents according to the notes.
	Not 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℃ Stored RNA in -20℃ or -70℃.
	RNA degrade during the extraction process.	Make extraction as fast as possible, centrifuge in low temprauture, treat RNA on ice.

Downstream RT-PCR failure	Forget to do step 11, or the adsorbing 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 adsorbing column carefully, or dry in the air, let the ethanol evaporate.