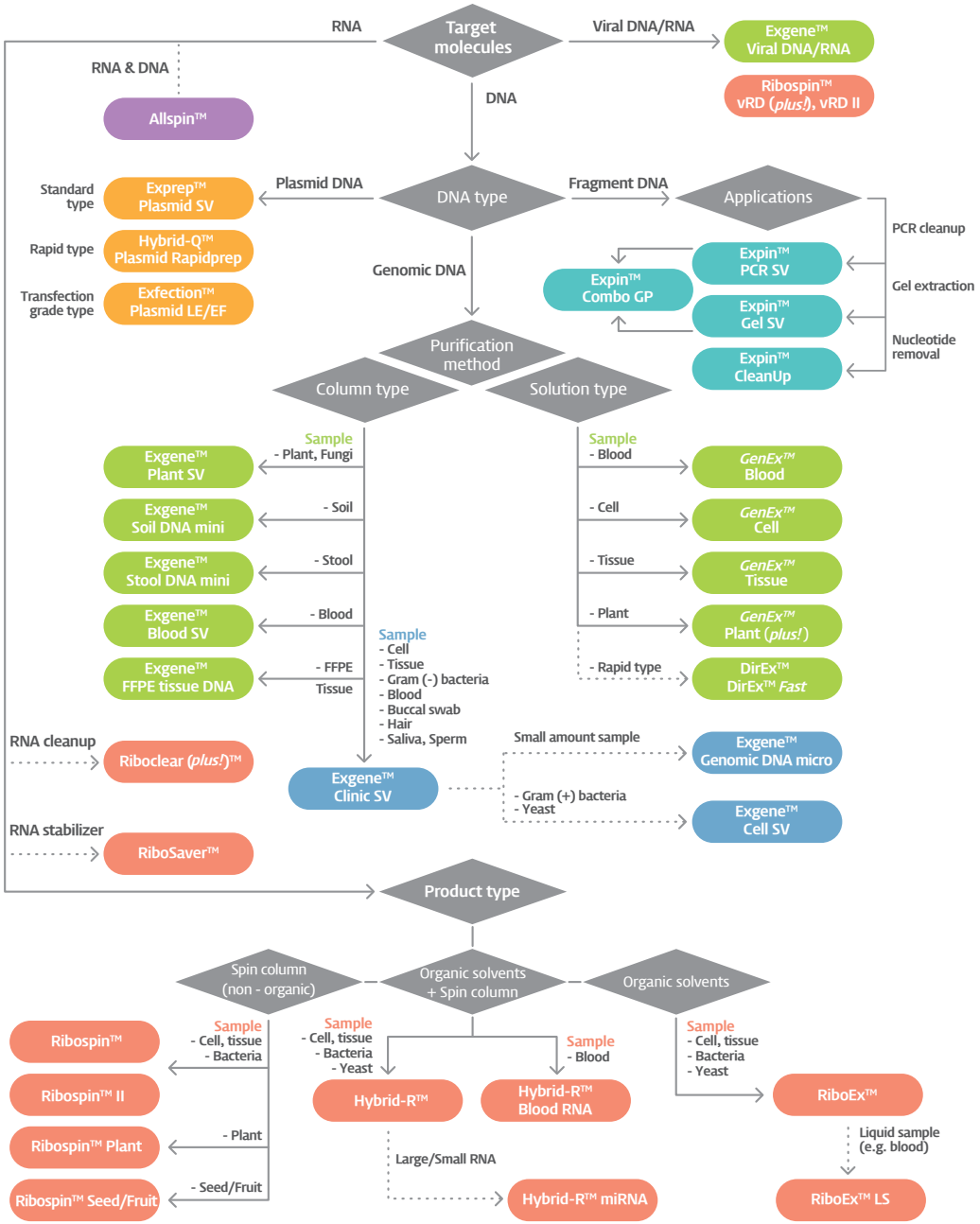
Selection Guide

Find GeneAll® Kit for successful experiments !



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Ver 2.0

GeneAll®

For research use only

Riboclear™ *plus!*

Cat. No. 313-150
Size: 50 prep

Kit Contents

Components	Quantity	Storage
DNase I	55 ul	-20 ℃
Buffer MS (concentrate) *	16 ml	Room temperature
Buffer RNW (concentrate) * †	12 ml	
Nuclease-free water	15 ml	
DNase I buffer (10 X)	1 ml	
Micro column type S (with collection tube)	50	
1.5 ml microcentrifuge tube	50	

** Before using for the first time, add absolute ethanol (ACS grade or better) into buffer MS and RNW as indicated on the bottle.*

† *Contains sodium azide as a preservative*

Product Specifications

Riboclear™ *plus!* Specifications	
Type	Spin
Maximum amount of starting samples	~ 100 ul
RNA recovery rate	~ 95%
Preparation time	~ 17 minutes
Maximum loading volume	~ 800 ul
Minimum elution volume	20 ul
Binding capacity	~ 100 ug

Quality Control

Riboclear™ *plus!* is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

Storage Conditions

Riboclear™ *plus!* should be stored at room temperature. But prolonged storage at high temperature over 30°C can reduce the performance of the kit. All components are stable for 1 year. Keep out of direct sunlight.

Precautions

The buffers included in Riboclear™ *plus!* contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice.
Buffer MS contains chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preventing RNase Contamination

RNase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

Product description

Riboclear™ *plus!* provides a convenient method for DNase I treatment and clean-up of total RNA. Riboclear™ *plus!* procedures employed the glassfiber membrane technology for the clean-up of total RNA, instead of conventional alcohol precipitation.

In this Riboclear™ *plus!* kit, especially, contaminated DNA in extracted total RNA can be removed by DNase I treatment prior to starting the procedure of RNA clean-up.

After the step for removal of contaminated DNA, RNA-containing samples mixed with buffer MS are applied to a micro spin column, followed by centrifugation. RNA binds to silica membrane while most of impurities pass through. The membrane is washed by buffer RNW for removal of some molecules bound nonspecifically. At last, pure RNA is eluted by Nuclease-free water. Riboclear™ *plus!* procedure should be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to contaminants, such as RNases, often found on general lab ware and dust. To ensure RNA-stability, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

Protocol of Riboclear™ *plus!*

<The procedure for removal of contaminated DNA>

1. **Prepare the mixture as below in a 1.5 ml microcentrifuge tube.**
   50 ul RNA eluate
   5 ul DNase I buffer (10 X)
   1 ul DNase I

   If the volume of your sample is more than 50 ul, adjust DNase I buffer to the volume of RNA eluate proportionally.

2. **Incubate the mixture at room temperature for 10 minutes.**

3. **Continue with "The procedure of RNA clean-up and concentration"**

<The procedure of RNA clean-up and concentration>

1. **Add 5 volumes of buffer MS to 1 volume of the sample and mix thoroughly.**
   For 50 ul reaction, add 250 ul of Buffer MS.
   * Do not centrifuge.

2. **Transfer the mixture to a micro spin column.**

3. **Centrifuge at ≥ 10,000 x g for 30 seconds.**
   Discard the pass-through and reinsert the micro spin column back into the collection tube.
   If the mixture volume is more than 700 ul, apply the mixture twice; apply 700 ul of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the micro spin column.

4. **Apply 700 ul of buffer RNW.**

5. **Centrifuge at ≥ 10,000 x g for 30 seconds.**
   Discard the pass-through and reinsert the micro spin column back into the collection tube.

6. **Centrifuge at ≥ 10,000 x g for an additional 1 minute to remove residual wash buffer.**
   Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

7. **Transfer the micro spin column to a new 1.5 ml microcentrifuge tube (provided).**

8. **Apply 50 ul of Nuclease-free water to the center of the membrane in the micro spin column. Let it stand for 1 minute, and centrifuge at ≥ 10,000 x g for 1 minute.**
   To obtain more concentrated RNA solution, apply 20 ul of Nuclease-free water. The yield can be significantly decreased if the volume of eluent is lower than 20 ul. Purified RNA can be stored at 4℃ for immediate analysis and stored at -70℃ for long-term storage.

Troubleshooting Guide

Facts	Possible causes	Suggestions
Poor quality and yield of RNA	Incorrect procedure	Buffer MS and samples should be mixed completely. Do not centrifuge after mix.
	Improper storage of kit	Store kit components at room temperature. Storage at low temperature may cause salt precipitation. Keep bottles tightly closed in order to avoid evaporation or contamination.
	Nuclease-free water applied incorrectly	Ensure that Nuclease-free water is applied to the center of membrane.
	Too much volume of Nuclease-free water	Reduce the volume of eluent.
Degradation of RNA	Contamination of RNase	RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation.
	Improper storage of RNA	Store isolated RNA at -70ºC, Do not store at -20ºC.
Genomic DNA contamination	Starting sample has high DNA mass	At step 1, 1 ul of DNase I can be used for upto 25 ug of DNA contaminants. Increase the DNase I upto 2 ul or decrease the starting sample down to 50 ul.
	DNase I not active	For prolonged activity, aliquot the DNase I into small portion. Do not freezing and thawing the aliquots several times.
RNA does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer RNW from micro spin column membrane, centrifuge again for complete removal of ethanol (step 6).