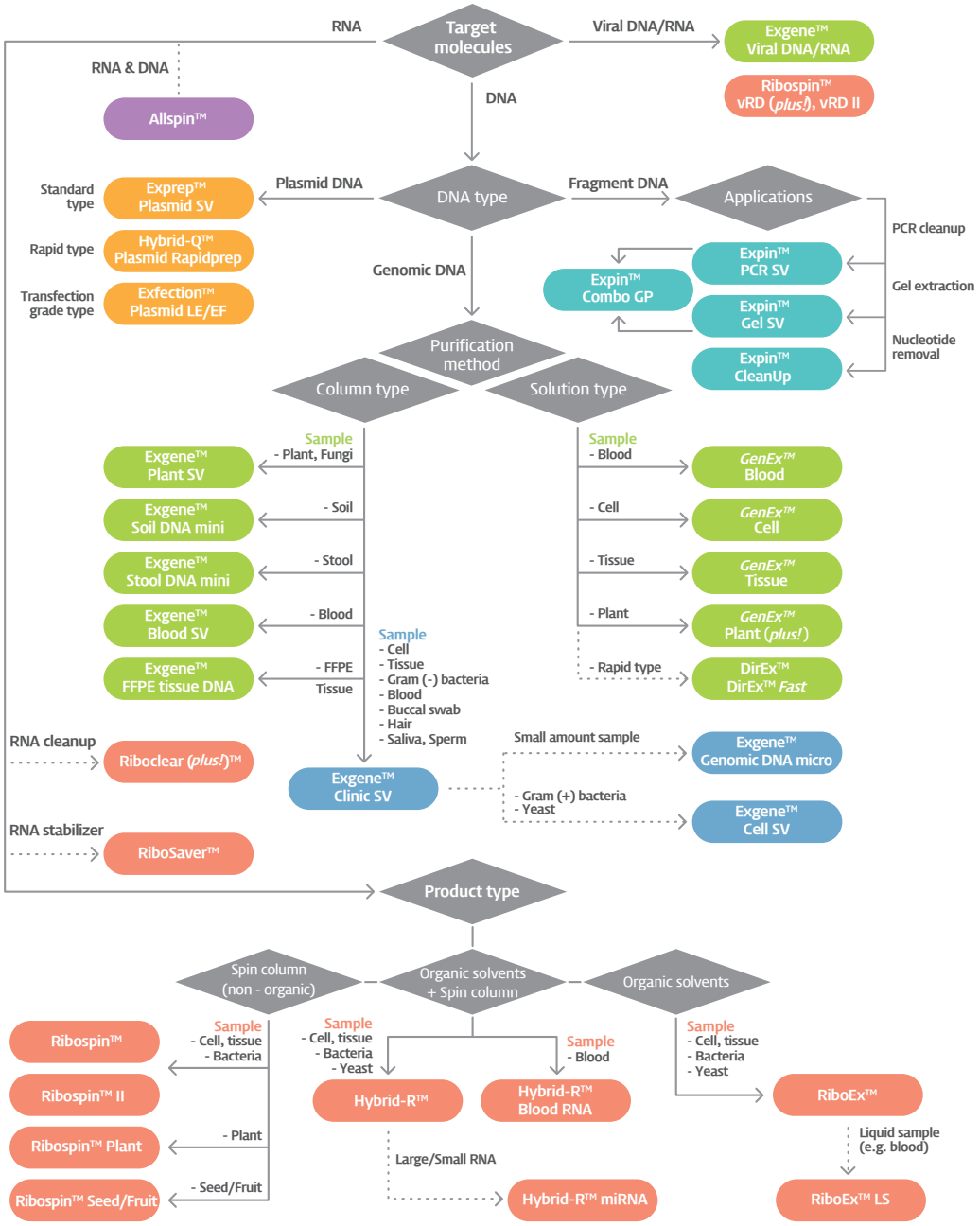
Selection Guide

Find GeneAll® Kit for successful experiments !



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Ribospin™ vRD *plus!*

Cat. No. 312-150
Size: 50 prep

Kit Contents

Components	Quantity	Storage
Buffer VL	30 ml	Room temperature (15 ~ 25°C)
Buffer RB1 (concentrate) *	8 ml	
Buffer RBW (concentrate) *	13 ml	
Buffer RNW (concentrate) * †	6 ml	
Nuclease-free water	15 ml	
Carrier RNA **	270 ug	
GeneAll® Column type V (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 RB1, RBW and RNW as indicated on the bottle.*
† Contains sodium azide as a preservative
*** Refer to page 3 for carrier RNA*

Product Specifications

Ribospin™ vRD *plus!* Specifications	
Type	Spin
Maximum volume of starting samples	300 ul / prep
Preparation time	~ 20 minutes
Maximum loading volume	750 ul
Minimum elution volume	30 ul

Quality Control

Ribospin™ vRD *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

Ribospin™ vRD *plus!* should be stored at room temperature (15 ~ 25°C). All components are stable for 1 year.

Precautions

The buffers included in Ribospin™ vRD *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 VL, RB1, and RNW contain 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

Ribospin™ vRD *plus!* provides a convenient method for isolation of RNA and DNA from cell-free fluid, cell-culture supernatant, plasma, serum, swab, urine, and virus-infected samples.

Ribospin™ vRD *plus!* procedures employed the glassfiber membrane technology for the fastest and the most convenient of high purity RNA and DNA isolation, instead of conventional alcohol precipitation or phenol/chloroform extraction.

Ribospin™ vRD *plus!* buffer system provides the effective binding condition of RNA and DNA to glassfiber membrane through mix with lysis and binding buffers. And then the impurities on the membrane are washed away by two different wash buffers. At last, pure RNA and DNA are eluted by Nuclease-free water. Whole procedure may take only 15 minutes and the eluate is suitable for PCR, RT-PCR, or any downstream application without further manipulation.

Ribospin™ vRD *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 labware 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 Ribospin™ vRD *plus!*

1. **Transfer upto 150 (300) ul sample (swab-storage media, cell-free fluid, cell-culture supernatant, plasma, serum, urine) in 1.5 ml microcentrifuge tube.**

2. **Add 250 (500) ul of buffer VL and 5 ul of carrier RNA to the tube and mix the sample by pipetting or vortexing.**
   Check buffer VL for precipitation. If happened, dissolve precipitate completely by incubation at 37°C or above.
   The volume of buffer VL can be adjusted in proportion to the volume of sample.
   For proper lysis, the complete mix of sample and buffer VL is essential.

3. **Incubate the lysate for 10 minutes at room temperature.**
   After this step, briefly centrifuge the tube to remove drops from the inside of the lid.

4. **Add 350 (700) ul of buffer RB1 to the lysate and mix thoroughly by inverting or vortexing.**
   The volume of buffer RB1 can be adjusted in proportion to the volume of lysate.
   * Do not centrifuge.

5. **Transfer upto 750 ul of the mixture to a mini spin column.**

6. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
   Discard the pass-through and reinsert the mini spin column back into the same tube.
   If the sample volume exceeds 750 ul, repeat step 5 ~ 6 with the remainder of the sample.

7. **Add 500 ul of buffer RBW to the mini spin column.**

8. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
   Discard the pass-through and reinsert the mini spin column back into the same tube.

9. **Add 500 ul of buffer RNW to the mini spin column.**

10. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
    Discard the pass-through and reinsert the mini spin column back into the same tube.

11. **Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer.**
    Transfer the mini spin column to a new 1.5 ml microcentrifuge tube (provided).
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

12. **Add 30 ~ 50 ul of Nuclease-free water to the center of the membrane in the mini spin column. Let it stand for 1 minute.**

13. **Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.**
    Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long-term storage.

Carrier RNA

This kit is provided with carrier RNA, which can be added to at lysis step if required. Carrier RNA enhances binding of nucleic acid to the mini spin column membrane, especially if there are very few target molecules in the sample.

For purification of nucleic acid from very small amounts of sample, we recommend adding carrier RNA at lysis step. To obtain a solution of 1 ug/ul, add 270 ul of Nuclease-free water to the tube containing 270 ug lyophilized carrier RNA. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C. Don't freeze-thaw the aliquots of carrier RNA more than 3 times. For one preparation, 5 ul of dissolved carrier RNA is required.

Troubleshooting Guide

Facts	Possible causes	Suggestions
Low yield	Poor quality of starting material	Repeated freezing and thawing should be avoided.
	Low concentration of virus in the sample	Use more sample. Concentrate the sample volume to 300 ul using a microconcentrator.
	Sample not homogenized completely	Be sure to incubate for 10 minutes at room temperature after lysis. For proper lysis, the complete mix of sample and buffer VL is essential.
	Incorrect elution conditions	Add Nuclease-free water to the center of the mini spin column membrane and perform incubation for 1 minute before centrifugation.
	Precipitation of buffer VL	Storage at low temperature may cause precipitation in buffer VL. For good result, any precipitate in the buffer should be dissolved completely by incubating the buffer at 37°C (or above) until it disappears.
	Degradation of RNA	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.
	Buffer RBW and RNW used in the wrong order	Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in buffer RNW from mini spin column membrane, centrifuge again for complete removal of ethanol (step 11).
	Buffer RBW and RNW used in the wrong order	Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.