TIANGEN®

Magnetic Viral DNA/RNA Kit

www.tiangen.com/en

This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

Magnetic Viral DNA/RNA Kit

Cat. no. 4992408/4992409

Kit Contents

Contents	4992408 (50 preps)	4992409 (200 preps)
Buffer RLCK	15 ml	60 ml
Buffer PWC	18 ml	80 ml
Buffer PWE	12 ml	50 ml
Carrier RNA	310 μg	2 × 310 μg
Proteinase K	1 ml	4 × 1 ml
MagAttract Suspension G	1 ml	4 × 1 ml
RNase-Free ddH$_2$O (Tube)	1 ml	2 × 1 ml
RNase-Free ddH$_2$O (Bottle)	15 ml	40 ml
Handbook	1	1

Storage Conditions:

All buffers can be stored for 15 months at room temperature (15-30°C). The lyophilized powder Carrier RNA can be stored at room temperature until the expiration date. Carrier RNA solution dissolved in RNase-Free ddH$_2$O should be frozen at -30~-15°C. Carrier RNA solution dissolved in Buffer RLCK can be stored at 2-8°C for up to 48 h. Please prepare the Carrier RNA solution right before use.

Introduction

The kit adopts magnetic beads with unique separation function and a unique buffer system to separate and purify high-quality viral DNA/RNA from serum, plasma, lymph, cell-free body fluid, cell culture supernatant, urine or various preservation solutions. Unique embedded magnetic beads have strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release adsorbed nucleic acid, thus achieving the purpose of fast separation and purification of nucleic acid. The whole process is safe and convenient. The extracted viral DNA/RNA has high yield, high purity, stable and reliable quality, and is especially suitable for automatic extraction of high-throughput workstations.

The nucleic acid purified by the kit is suitable for various conventional operations, including various downstream experiments such as RT-PCR, fluorescence quantitative PCR, etc.

Features

Easy and fast: High-quality viral DNA or RNA can be obtained within 1 hr.

High Throughput: It can be combined with magnetic rod based and pipetting based automatic instruments to carry out high throughput extraction experiments.

Safe and non-toxic: No organic reagents such as phenol/chloroform are needed.

Important Notes Before Starting

1. This product is suitable for manual extraction or automatic instrument integration.
2. Self-prepared reagent: 96-100% ethanol, isopropanol.
3. Avoid repeated freezing and thawing of the sample, otherwise the extracted nucleic acid fragments will be smaller and the extraction amount will be reduced.
4. If there is precipitation in Buffer RLCK, it can be redissolved in 37°C water bath, and please use after shaking to mix thoroughly.

Preparation of Carrier RNA solution

- **Carrier RNA solution:** Add 310 μl of RNase-Free ddH_2O to a tube filled with 310 μg of Carrier RNA lyophilized powder. Completely dissolve the Carrier RNA to obtain a solution with a final concentration of 1 μg/μl, and aliquot the solution into RNase-free centrifuge tubes according to experimental needs and store at -30~-15°C. When in use, take out the corresponding solution according to the times of extraction. Avoid repeated freezing and thawing of the solution, and the freezing and thawing should not exceed 3 times.

  Note: Carrier RNA freeze-dried powder cannot be directly dissolved in Buffer RLCK, but must be dissolved in RNase-Free ddH_2O before being dissolved in Buffer RLCK.

- **Carrier RNA working solution:** Calculate the volume of the required Buffer RLCK and Carrier RNA solution according to the number of samples (see Table 1, please prepare according to the proportion of 2.8 μl Carrier RNA per 310 μl RLCK), and mix Buffer RLCK and Carrier RNA solution to obtain Carrier RNA working solution. In order to avoid foaming of the solution, do not vortex the solution.

Table 1. Preparation of Carrier RNA Working Solution

No. of reactions	RLCK (ml)	Carrier RNA solution(μl)	No. of reactions	RLCK (ml)	Carrier RNA solution(μl)
1	0.31	2.8	13	4.03	36.4
2	0.62	5.6	14	4.34	39.2
3	0.93	8.4	15	4.65	42
4	1.24	11.2	16	4.96	44.8
5	1.55	14	17	5.27	47.6
6	1.86	16.8	18	5.58	50.4
7	2.17	19.6	19	5.89	53.2
8	2.48	22.4	20	6.2	56
9	2.79	25.2	21	6.51	58.8
10	3.1	28	22	6.82	61.6
11	3.41	30.8	23	7.13	64.4
12	3.72	33.6	24	7.44	67.2

Protocol

I. Manual protocol

Before starting, please add isopropanol to Buffer RLCK according to the instructions on the label.

Please add 96-100% ethanol into Buffer PWC and PWE according to the instructions on the label.

1. Transfer 200 μl of plasma/serum/lymph (sample needs to be balanced to room temperature) to a 1.5 ml centrifuge tube (self-provided).
2. Add 15 μl of MagAttract Suspension G to the centrifuge tube.

   Note: Before adding to the tube, vortex to mix thoroughly and completely resuspend the beads.
3. Add 20 μl Proteinase K to the tube.
4. Add 300 μl Carrier RNA working solution (mixture of Buffer RLCK (please check whether isopropanol has been added before use) and Carrier RNA solution. The preparation method is as shown in Table 1) to the centrifuge tube. Vortex for 10 sec to mix.

   Note: When the number of samples is relatively large, premix 20 μl Proteinase K with every 300 μl Carrier RNA working solution. After mixing, the dosage for each sample is 320 μl. The mixture should be kept at room temperature for no more than 1 h.
5. Incubate at room temperature for 10 min, and turn the tube upside down for 10 sec every 3 min during the incubation for a better binding effect. After the incubation, briefly centrifuge the tube to remove the drops from the cap and tube wall.
6. Place the centrifuge tube on a magnetic stand and let it stand for 1 min. When the magnetic beads are completely attached, carefully remove the supernatant with a pipette.
7. Remove the centrifuge tube from the magnetic stand, add 500 μl of Buffer PWC **(ensure that 96-100% ethanol is added before use)**, and vortex for 1 min to mix.
8. Place the centrifuge tube on the magnetic stand and let stand for 1 min. When the magnetic beads are completely attached, carefully remove the supernatant with a pipette.
9. Remove the centrifuge tube from the magnetic stand, add 500 μl of Buffer PWE **(ensure that 96-100% ethanol is added before use)**, and vortex for 1 min to mix.

10. Place the centrifuge tube on the magnetic stand and leave it untouched for 1 min. When the magnetic beads are completely attached, carefully remove the supernatant with a pipette.
11. Repeat step 9 and 10 once.
12. Place the centrifuge tube on the magnetic stand and dry at 56°C for 5-10 min.

    Note: The ethanol residue will inhibit the subsequent enzyme reaction, so make sure the ethanol volatilizes completely when drying. However, do not over-dry, or it will be difficult to elute the nucleic acids.
13. Remove the centrifuge tube from the magnetic stand, add 100 μl RNase-free ddH_2O, and shake at 56°C for 5 min.
14. Place the centrifuge tube on the magnetic stand and let stand for 2 min. After the magnetic beads are completely attached, carefully transfer the nucleic acid solution to a new centrifuge tube (self-provided) and store it under appropriate conditions.

II. Automatic protocol (KingFisher Flex)

Before starting, please add isopropanol to Buffer RLCK according to the instructions on the label.

Please add 96-100% ethanol into Buffer PWC and PWE according to the instructions on the label.

1. Add 200 μl of plasma/serum/lymph to each well of a 96-well plate (self-provided) (sample needs to be balanced to room temperature).
2. Add 15 μl of MagAttract Suspension G to each well (mix the magnetic beads uniformly by pipetting or vortex oscillation before use).
3. Add 20 μl Proteinase K to each well.
4. Add 300 μl Carrier RNA working solution (mixture of Buffer RLCK (please check whether isopropanol has been added before use) and Carrier RNA solution. The preparation method is as shown in Table 1) into each well and vortex for 10 sec to mix.

   Note: When the number of samples is relatively large, premix 20 μl Proteinase K with every 300 μl Carrier RNA working solution. After mixing, the dosage for each sample is 320 μl. The mixture should be kept at room temperature for no more than 1 hr.
5. Transfer samples and reagents to 96-deep-well Plate according to the following table, and mark the name of the plate with a label pen.

Plate type	96-well plate	Reagent and dosage
Elution	Deep-well plate	RNase-Free ddH_2O: 100 μl
Wash 2_2	Deep-well plate	PWE **(ensure that ethanol is added before use)**: 500 μl
Wash 2_1	Deep-well plate	PWE **(ensure that ethanol is added before use)**: 500 μl
Wash 1	Deep-well plate	PWC **(ensure that ethanol is added before use)**: 500 μl
Sample	Deep-well plate	Sample: 200 μl
		Carrier RNA working solution: 300 μl
		Proteinase K: 20 μl
		MagAttract Suspension G: 15 μl
Tip plate	Deep-well plate	Deep Well 96 Tip Comb

6. Start KingFisher BindIt 3.2 program and import Pure Viral DNA_RNA Kit. bdz program.
7. The program is completed in about 33 min.
8. Take out DNA or RNA samples, seal with sealing film and store at -80°C.

   Note: If you need to combine with other automatic nucleic acid extractor by magnetic rod method or pipetting method, please contact TIANGEN.