Magnetic Viral DNA/RNA Kit

Product Name: Magnetic Viral DNA/RNA Kit

Package Size: 50/200 preps/kit

Application: Highly efficient purification of viral nucleic acid from plasma, serum, urine, hydrothorax, cerebrospinal fluid, saliva, swab, stool, tissue, sputum, alveolar lavage fluid, environmental swab samples, etc.

Principle: After the samples containing the target nucleic acid are lysed by the lysis buffer, high purity nucleic acid is obtained by washing, eluting and purifying process based on the principle of specific and efficient combination of magnetic beads and nucleic acid.

Description: 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 regular operations, including various downstream experiments such as RT-PCR, real time qPCR, etc.

Product Components:

This product consists of the following components:

Reagent Name	CDP438-01 50 preps	CDP438-02 200 preps	CDP438-T3E 200 preps	CDP438-T4A 200 preps
Buffer RLCK	15 ml	60 ml	-	-
Buffer GHH	-	-	4×35 ml	-
Buffer RLCA	-	-	-	60 ml
Buffer PWC	18 ml	80 ml	-	80 ml
Buffer PD	-	-	4×35 ml	-
Buffer PWE	12 ml	50 ml	4×10 ml	50 ml
Carrier RNA	310 μg	2×310 μg	310 μg	-
Proteinase K	1 ml	4×1 ml	-	4×1 ml
RNase-Free ddH_2O(Tube)	1 ml	2×1 ml	-	-
RNase-Free ddH_2O(Bottled)	15 ml	40 ml	4×5 ml	40 ml
MagAttract Suspension G	1 ml	4×1 ml	-	-
MagAttract Suspension FHE	-	-	4×1.2 ml	-
MagAttract Suspension H	-	-	-	4×1 ml
Handbook	1	1	1	1

Storage and Shelf Life:

All buffers can be stored at room temperature (15-30°C). The shelf life is 12 months. For long term storage, please store at 2-8°C.

The lyophilized powder Carrier RNA can be stored at room temperature until the expiration date. Carrier RNA solution dissolved in RNase-Free ddH_2O should be frozen at -20°C. Carrier RNA solution dissolved in Buffer RLCK/GHH can be stored at 2-8°C for up to 48 hours. Please prepare the Carrier RNA solution right before use.

Important Notes: Please read these notes before using this kit.

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 yield will be reduced.
4. If there is precipitation in Buffer RLCK/GHH/RLCA, it can be redissolved in 37°C water bath, and please use after shaking to mix thoroughly.

Protocol

I. Sample Treatment

a) Plasma/serum/urine/hydrothorax/cerebrospinal fluid/saliva samples

Equilibrate the sample to room temperature, and directly proceed to the next step. If the saliva sample of animal contains food residues, centrifuge at 12,000 rpm (13,400 × g) for 2 min to collect the saliva.

b) Swab samples

Dry swab samples: Add in certain volume of normal saline *(the normal saline should immerse the swab)*. Mix thoroughly by vortex.

Swab samples in preservation solution: Mix thoroughly by vortex and proceed to the next step.

c) Fecal samples

Samples without preservation solution: Suspend the fecal sample in 5 times volume of normal saline. After thorough mixing by vortex, centrifuge at 12,000 rpm (13,400 × g) for 2 min.

Samples with preservation solution: Mix thoroughly by vortex, centrifuge at 12,000 rpm (13,400 × g) for 2 min and proceed to the next step.

d) Tissue samples

Add appropriate volume of PBS or normal saline to the tissue block, homogenate the tissue, then centrifuge at 12,000 rpm (13,400 × g) for 2 min and take the supernatant to proceed to the next step.

e) Sputum/alveolar lavage fluid samples

If the sputum is viscous, add 30 μl Buffer ST *(Cat# RK247, not supplied)* to the sample in advance for liquefaction, and then proceed to the next step.

f) Environmental swab samples

Use the sterile cotton swab soaked in sterile normal

saline to evenly wipe horizontally and vertically in the most likely contact place (such as the desk board, door handle, work clothes, etc.) within the range of 10 cm × 10 cm for 5 times. Rotate the swab when wiping. After cutting off the hand-contact part, put the swab into 10 ml sterile normal saline or commercial sampling tube. Before the extraction,thoroughly mix by vortex, and proceed to the next step.

II. DNA/RNA Extraction

1. CDP438-01/CDP438-02

Please ensure that Buffer PWC and Buffer PWE have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle.

Please ensure that Buffer RLCK have been prepared with appropriate volume of isopropanol as indicated on the bottle.

i. Manual protocol

1) Transfer 200 µl sample *(sample needs to be equilibrated to room temperature)* to a 1.5 ml centrifuge tube *(self-prepared)*.

2) Add 15 µl of MagAttract Suspension G to the centrifuge tube.

   Note: Before adding to the tube, vortex to mix thoroughly and completely suspend 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 to Buffer RLCK 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 hour.

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 ethanol (96-100%) 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 ethanol (96-100%) 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-prepared)* and store it under appropriate conditions.

ii. Automatic protocol (KingFisher Flex)

1) Add 200 µl sample to each well of a 96-well plate *(self-prepared) (sample needs to be equilibrated 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 *(please check whether isopropanol has been added to Buffer RLCK before use. 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 hour.

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 (check if ethanol is added before use): 500 µl
Wash 2_1	Deep-well plate	PWE (check if ethanol is added before use): 500 µl
Wash 1	Deep-well plate	PWC(check if ethanol is added before use): 500 µl
Sample	Deep-well plate	Sample: 200 µl
		Carrier RNA solution: 300 µl
		Proteinase K: 20 µl
		MagAttract Suspension G: 15 µl
Comb plate	Deep-well plate	Comb

6) Start KingFisher BindIt 3.4 program and import Pure Viral DNA_RNA Kit.bdz program.

7) Take out DNA or RNA samples, seal with sealing film and store at -20°C or -80°C.

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

2.CDP438-T3E:

Please ensure that Buffer PWE have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle.

i. Manual protocol

1) Transfer 400 µl sample *(sample needs to be equilibrated to room temperature)* to a 1.5 ml centrifuge tube *(self-prepared)*.

2) Add 20 µl of MagAttract Suspension FHE to the centrifuge tube.

Note: Before adding to the tube, vortex to mix thoroughly and completely suspend the beads.

3) Add 600 µl Buffer GHH and 1 µl Carrier RNA solution to the centrifuge tube. Vortex for 10 sec to mix.

4) 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.

5) 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.

6) Remove the centrifuge tube from the magnetic stand, add 500 µl of Buffer PD, and mix well for 1 min.

7) 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.

8) Remove the centrifuge tube from the magnetic stand, add 500 µl of Buffer PD, and mix well for 1 min.

9) 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.

10) Remove the centrifuge tube from the magnetic stand, add 500 µl of Buffer PWE *(ensure that ethanol (96-100%) is added before use)*, and mix well for 1 min.

11) 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.

12) Repeat step 10) and 11) once.

13) Place the centrifuge tube on the magnetic stand and air-dry at room temperature 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.

14) Remove the centrifuge tube from the magnetic stand, add 100 µl RNase-free ddH_2O, and shake at 25°C for 5 min.

15) 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-prepared)* and store it under appropriate conditions.

ii. Automatic protocol (KingFisher Flex)

1) Add 400 µl sample to each well of a 96-well plate *(self-prepared) (sample needs to be equilibrated to room temperature)*.

2) Add 20 µl of MagAttract Suspension FHE to each well *(mix the magnetic beads uniformly by pipetting or vortex oscillation before use)*.

3) Add 600 µl Buffer GHH and 1 µl Carrier RNA solution into each well and vortex for 10 sec to mix.

4) 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 (check if ethanol is added before use): 500 µl
Wash 2_1	Deep-well plate	PWE (check if ethanol is added before use): 500 µl
Wash 1_2	Deep-well plate	PD: 500 µl
Wash 1_1	Deep-well plate	PD: 500 µl
Sample	Deep-well plate	Sample: 400 µl
		Carrier RNA solution: 600 µl
		MagAttract Suspension FHE:10 µl
Comb plate	Deep-well plate	Comb

5) Start KingFisher BindIt 3.4 program and import Pure Viral DNA_RNA Kit.bdz program.

6) Take out DNA or RNA samples, seal with sealing film and store at -20°C or -80°C.

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

3.CDP438-T4A:

Please ensure that Buffer PWE and Buffer PWC have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle.

Please ensure that Buffer RLCA have been prepared with appropriate volume of isopropanol as indicated on the bottle.

i. Manual protocol

1) Transfer 200 µl sample *(sample needs to be equilibrated to room temperature)* to a 1.5 ml centrifuge tube *(self-prepared)*.

2) Add 20 µl of MagAttract Suspension H to the centrifuge tube.

Note: Before adding to the tube, vortex to mix thoroughly and completely suspend the beads.

3) Add 20 µl Proteinase K to the centrifuge tube.

4) Add 300 µl Buffer RLCA *(ensure isopropanol has been*

added before use). Vortex for 10 sec to mix.

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 thatethanol (96-100%) is added before use)*, and mix well for 1 min.

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 thatethanol (96-100%) is added before use)*, and mix well for 1 min.

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 air-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 25°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-prepared)* and store it under appropriate conditions.

ii. Automatic protocol (KingFisher Flex)

1) 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 1	Deep-well plate	PWC (check if ethanol is added before use): 500 µl
Wash 2	Deep-well plate	PWE (check if ethanol is added before use): 500 µl
		MagAttract Suspension H: 20 µl
		Magnetic rod comb
Sample	Deep-well plate	Sample: 200 µl
		Buffer RLCA: 300 µl
		Proteinase K: 20 µl
Comb plate	Deep-well plate	Comb

2) Add 20 µl Proteinase K and 200 µl sample in the 96-deep-well-plate *(sample plate)(sample needs to be equilibrated to room temperature)*.

3) Start KingFisher BindIt 3.4 program and import Pure Viral DNA_RNA Kit.bdz program.

4) Take out DNA or RNA samples, seal with sealing film and store at -20°C or -80°C .

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

Preparation of Carrier RNA solutions

- Carrier RNA solution: Add 310 µl RNase-Free ddH_2O to the tube containing 310 µg lyophilized Carrier RNA to obtain a solution of 1 µg/µl. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.

  Note: Lyophilized Carrier RNA should not be dissolved directly in Buffer RLCK/GHH. It must firstly be dissolved in RNase-Free ddH_2O and then added to Buffer RLCK/GHH.

- Carrier RNA working solution: Calculate the volume of Buffer RLCK/GHH and the Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from table 1 *(please prepare according to the proportion of 2.8 µl Carrier RNA per 310 µl RLCK or 1 µl Carrier RNA per 600 µl GHH)*.

Table 1 Preparation of Carrier RNA Working Solution

No. Sample	CDP438-01/02		CDP438-T3E	
	Vol. Buffer RLCK (ml)	Vol. Carrier RNA /RNase-Free ddH_2O (µl)	Vol. Carrier GHH (ml)	Vol. Carrier RNA /RNase-Free ddH_2O (µl)
1	0.31	2.8	0.6	1
2	0.62	5.6	1.2	2
3	0.93	8.4	1.8	3
4	1.24	11.2	2.4	4
5	1.55	14	3	5
6	1.86	16.8	3.6	6
7	2.17	19.6	4.2	7
8	2.48	22.4	4.8	8
9	2.79	25.2	5.4	9
10	3.1	28	6	10
11	3.41	30.8	6.6	11
12	3.72	33.6	7.2	12
13	4.03	36.4	7.8	13
14	4.34	39.2	8.4	14
15	4.65	42	9	15
16	4.96	44.8	9.6	16
17	5.27	47.6	10.2	17
18	5.58	50.4	10.8	18
19	5.89	53.2	11.4	19
20	6.2	56	12	20
21	6.51	58.8	12.6	21
22	6.82	61.6	13.2	22
23	7.13	64.4	13.8	23
24	7.44	67.2	14.4	24

Note: Mix Buffer RLCK/GHH with Carrier RNA solution by inverting the tube. Don't vortex to avoid bubbling.

Limitation of the Detection Method

This kit can not be used independently, it needs to be used together with a centrifuge.

Manufacturer

Manufacturer name:
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