TIANamp Virus RNA Kit

Product Name: TIANamp Virus RNA Kit

Package Size: 50 preps/kit

Application: For purification of virus RNA 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 silica gel membrane and nucleic acid.

Description: The TIANamp Virus RNA Kit provides a fast, simple, and cost-effective viral RNA miniprep method and it is suitable for virus RNA from plasma, serum, and cell-free body fluids. TIANamp Virus DNA/RNA Kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Viral RNA purified with TIANamp Virus RNA Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required. The purified RNA is ready for use in downstream applications such as enzymatic reactions, RT-PCR, Southern blot and so on.

Product Components:

This product consists of the following components:

Reagent Name	CDP315-R	CDP315-T8	CDP315-T10
Buffer RL	30 ml	-	-
Buffer RLC	-	35 ml	35 ml
Buffer GD	13 ml	13 ml	13 ml
Buffer RW	12 ml	12 ml	12 ml
RNase-Free ddH_2O(Bottled)	15 ml	15 ml	15 ml
Proteinase K	-	1 ml	1 ml
Carrier RNA	310 μg	-	-
RNase-Free ddH_2O(Tube)	1 ml	-	-
RNase-Free Spin Column CR2 set	50 sets	-	-
RNase-Free Spin Column CA4	-	50/bag	-
RNase-Free Spin Column CR4	-	-	50/bag
Collection Tube 2 ml	-	50/bag	50/bag
RNase-Free Centrifuge Tubes 1.5 ml	50/bag	50/bag	50/bag
Handbook	1	1	1

Storage and Shelf Life

All buffers can be stored at room temperature (15-30°C). The shelf life is 12 months.

Lyophilized Carrier RNA is stable for up to one year at room temperature (15-30°C). Carrier RNA can only be dissolved in RNase-Free ddH_2O; dissolved Carrier RNA should be immediately added to Buffer RL as described in this handbook. This solution should be prepared fresh, and is stable at 2-8°C for up to 48 hours. Unused portions of Carrier RNA dissolved in RNase-Free ddH_2O should be frozen in aliquots at -20°C.

Important Notes: Please read these notes before using this kit

1. All centrifuge steps should be carried out at room temperature (15-30°C).
2. Equilibrate the sample to room temperature before use.
3. RNase-Free Centrifuge tubes 1.5 ml are used in the elution step. Others are not supplied.

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

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

1.Sample preparation

CDP315-R:

1) Add 560 µl of Carrier RNA working solution *(mixture of Buffer RL and Carrier RNA solution, the preparation method is shown in Table 1 or calculated according to the formula)* into a clean 1.5 ml centrifuge tube *(self prepared)*.

2) Add 140 µl sample *(equilibrate the samples to room temperature (15-30°C))* to the Buffer RL-Carrier RNA in the centrifuge tube. Mix by pulse-vortex for 15 sec. To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Carrier RNA working solution to yield a homogeneous solution.

   Note: If the sample volume is larger than 140 µl, increase the amount of Buffer RL-Carrier RNA proportionally.

3) Incubate at room temperature (15-30°C) for 10 min.

4) Briefly centrifuge the tube to remove drops from the inside of the lid.

5) Add 560 µl of ethanol (96-100%) to the sample, and mix by pulse-vortex for 15 sec.

   Note: Cool ethanol on ice before use if the room temperature is more than 25°C.

CDP315-T8:

1) Add 300 µl sample into the centrifuge tube *(equilibrate the samples to room temperature (15-30°C))*.

2) Add 20 µl Proteinase K and 600 µl Buffer RLC. Vortex to mix and incubate at 56°C for 10 min.

3) Briefly centrifuge the tube to remove drops from the inside of the lid.

4) Cool the solution to room temperature, then add 600 µl isopropanol or ethanol (96-100%) to the tube, and vortex for 2 min to mix.

   Note: Cool ethanol on ice before use if the room temperature is more than 25°C.

CDP315-T10:

1) Add 500 µl sample into the centrifuge tube *(equilibrate the samples to room temperature (15-30°C))*.

2) Add 20 µl Proteinase K and 500 µl Buffer RLC. Vortex to mix and incubate at 75°C for 10 min.

3) Briefly centrifuge the tube to remove drops from the inside of the lid.

4) Cool the solution to room temperature, then add 500 µl isopropanol or ethanol (96-100%) to the tube, and vortex for 2 min to mix.

   Note: Cool ethanol on ice before use if the room temperature is more than 25°C.

2. Briefly centrifuge the tube to remove drops from inside the lid.

3. Carefully transfer the lysate *(including any precipitates that may have formed)* to the RNase-Free Spin Column CR2 (CDP315-R)/CA4 (CDP315-T8)/CR4 (CDP315-T10) *(the spin column is placed in a 2 ml RNase-Free Collection Tube)* without wetting the rim. Close the cap and centrifuge at 12,000 rpm (~13,400×g) for 1 min. Discard the filtrate; place the RNase-Free Spin Column in the same collection tube.

   Note: If the lysate has not completely passed through the RNase-Free Spin Column after centrifugation, centrifuge again until the RNase-Free Spin Column is empty.

4. Repeat step 3 with the remaining lysate in the centrifuge tube.

5. Carefully open the RNase-Free Spin Column, and add 500 µl of Buffer GD *(Ensure that ethanol (96-100%) has been added before use)* without wetting the rim. Close the cap and centrifuge at 12,000 rpm (~13,400×g) for 1 min. Discard the filtrate and place the RNase-Free Spin Column in the same collection tube.

6. Carefully open the RNase-Free Spin Column, and add 500 µl of Buffer RW *(Ensure that ethanol (96-100%) has been added before use)* without wetting the rim. Close the cap and centrifuge at 12,000 rpm (~13,400×g) for 1 min. Discard the filtrate and place the RNase-Free Spin Column in the same collection tube.

7. Repeat step 6.

8. Place the RNase-Free Spin Column in the same collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 3 min to dry the membrane completely.

   Note: Ethanol carryover into the eluate may cause problems in downstream applicaitons.

9. *Optional: Place the RNase-Free Spin Column into the same 2 ml collection tube (not provided), open the lid, and incubate at room temperature (15-30°C) for 3 min to dry the membrane completely.*

10. Place the RNase-Free Spin Column in a clean 1.5 ml RNase-Free Centrifuge Tube, and discard the collection tube with the filtrate. Carefullly open the lid of the RNase-Free Spin Column, and apply 30-150 µl of RNase-Free ddH_2O to the center of the membrane. Close the lid and incubate at room temperature (15-30°C) for 5 min. Centrifuge at 12,000 rpm (~13,400×g) for 1 min.

    Note: Ensure that the elution buffer (RNase-Free ddH_2O) is equilibrated to room temperature (15-30°C) before use. If elution is done in small volumes (<50 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA. Adjust the volume of elution buffer according to the requirements of specific experiments. Incubate at room temperature (15-30°C) for 5 min to increase the RNA yield after RNase-Free ddH_2O is added into the RNase-Free Spin Column.

Preparation of Carrier RNA solutions

- 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.
- Lyophilized Carrier RNA should not be dissolved in Buffer RL directly. It must firstly be dissolved in RNase-Free ddH_2O and then added to Buffer RL.
- Carrier RNA working solution: Calculate the volume of Buffer RL and the Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from table 1. For larger numbers of samples, volumes can be calculated using the following sample calculation:

  n x 0.56 ml = y ml

  y ml x 10 µl/ml = z µl

  n = number of samples to be processed simultaneously

  y = calculated volume of Buffer RL

  z = volume of Carrier RNA/ RNase-Free ddH_2O to add to Buffer RL

Table 1 Volumes of Buffer RL and Carrier RNA/ RNase-Free ddH_2O mix required for the carrier RNA working solution

No. Sample	Vol. Buffer RL (ml)	Vol. CarrierRNA/ RNase-Free ddH_2O (µl)
1	0.56	5.6
2	1.12	11.2
3	1.68	16.8
4	2.24	22.4
5	2.80	28.0
6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0
11	6.16	61.6
12	6.72	67.2
13	7.28	72.8
14	7.84	78.4
15	8.40	84.0
16	8.96	89.6
17	9.52	95.2
18	10.08	100.8
19	10.64	106.4
20	11.20	112.0
21	11.76	117.6
22	12.32	123.2
23	12.88	128.8
24	13.44	134.4

Note: Mix Buffer RL 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:
TIANGEN BIOTECH (BEIJING) CO., LTD.

Registered and production address:
Building 5, No. 86, Shuangying West Road, Science and Technology Park, Changping District, Beijing

Post code: 102299

Contact Number: 800-990-6057