TIANamp Virus RNA Kit

For purification of viral RNA from plasma, serum, cell-free body fluids, cell-culture supernatants

TIANGEN®

www.tiangen.com/en

TIANamp Virus RNA Kit

(Spin Column)

Cat. no. 4992286

Kit Contents

Contents	4992286 50 preps
Buffer RL	30 ml
Buffer GD	13 ml
Buffer RW	12 ml
Carrier RNA	310 μg
RNase-Free ddH_2O	1 ml
RNase-Free ddH_2O	15 ml
RNase-Free Columns CR2 set	50
RNase-Free Centrifuge Tubes (1.5 ml)	50
Handbook	1

Storage

1.All buffers can be stored at room temperature (15-25°C).
2.Lyophilized Carrier RNA is stable for up to one year at room temperature (15-25°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.

Introduction

TIANamp Virus RNA Kit provides a fast and convenient format to obtain purified viral RNA for reliable use in amplification technologies. It is suitable for purification of viral RNA from plasma, serum, and cell-free body fluids and the procedure is optimized for use with 140-560 µl samples. Addition of Carrier RNA is used for isolating viral RNA from tiny amount of sample. TIANamp Virus 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 in downstream applications such as enzymatic reactions, PCR, southern blot and so on.

Important notes

1. All protocol steps should be carried out at room temperature (15-25°C).
2. Equilibrate the samples to room temperature.
3. RNase-Free Centrifuge tubes 1.5 ml are used in step 13. Others are not supplied.
4. **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/ 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. Carrier RNA/ 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.

Protocol

1. Please add ethanol (96-100%) to Buffer GD and RW before use, the volume as described on the bottle.

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

2. Add 140 µl plasma, serum or cell-free body fluid (Equilibrate the samples to room temperature (15-25°C)) to the Buffer RL-Carrier RNA in the centrifuge tube. Mix by pulse-vortex for 15 s. To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer RL to yield a homogeneous solution.
3. Incubate at room temperature (15-25°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 s.

   Note: Cool ethanol (96-100%) on ice before use if the room temperature is more than 25°C.

6. Briefly centrifuge the tube to remove drops from inside the lid.
7. Carefully transfer the 630 µl lysate onto the RNase-Free Spin Column CR2 in a 2 ml RNase-Free Collection Tube without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 × g) for 1 min. Discard the filtrate; place the RNase-Free Spin Column CR2 in the same collection tube.

   Note: If the lysate has not completely passed through the RNase-Free Spin Column CR2 after centrifugation, centrifuge again at higher speed until the RNase-Free Spin Column CR2 is empty.

8. Repeat step 7.
9. Carefully open the RNase-Free Spin Column CR2, 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 8,000 rpm (~6,000 × g) for 1 min. Discard the filtrate and place the RNase-Free Spin Column CR2 in the same collection tube.
10. Carefully open the RNase-Free Spin Column CR2, and add 500 µl Buffer RW **(Ensure that ethanol (96-100%) has been added before use)** without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 × g) for 1 min. Discard the filtrate and place the RNase-Free Spin

Column CR2 in the same collection tube.

11. Place the RNase-Free Spin Column CR2 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 applications.

12. *Optional: Place the RNase-Free Spin Column CR2 into the same 2 ml collection tube (not provided), open the lid, and incubate at room temperature (15-25°C) for 3 min to dry the membrane completely.*

13. Place the RNase-Free Spin Column CR2 in a clean 1.5 ml RNase-Free Centrifuge Tube, and discard the collection tube with the filtrate. Carefully open the lid of the RNase-Free Spin Column CR2, and apply 60 µl of RNase-Free ddH_2O to the center of the membrane. Close the lid and incubate at room temperature (15-25°C) for 5 min. Centrifuge at 8,000 rpm (~6,000 × g) for 1 min.

    Note: Ensure that the elution buffer (RNase-Free ddH_2O) is equilibrated to room temperature (15-25°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 (15-25°C) for 5 min to increase the RNA yield after RNase-Free ddH_2O is added into the RNase-Free Spin Column CR2.