TIANGEN®

# TIANamp Virus DNA/RNA Kit

For purification of Virus DNA/RNA from plasma, serum, and cell-free body fluids

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# TIANamp Virus DNA/RNA Kit

**(Spin Column)**

**Cat. no. 4992285**

## Kit Contents

| Contents | 4992285<br>50 preps |
|---|---|
| Buffer GB | 15 ml |
| Buffer GD | 13 ml |
| Buffer PW | 15 ml |
| RNase-Free dd$H_2O$ | 15 ml |
| Proteinase K | 1 ml |
| Carrier RNA | 310 μg |
| RNase-Free dd$H_2O$ | 1 ml |
| RNase-Free Columns CR2 set | 50 |
| RNase-Free Centrifuge Tubes 1.5 ml | 50 |
| Handbook | 1 |

## Storage

TIANamp Virus DNA/RNA Kit can be stored at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use. Carrier RNA can only be dissolved in RNase-Free dd$H_2O$; dissolved Carrier RNA should be stored at -30~-15°C.

## Introduction

The TIANamp Virus DNA/RNA Kit provides a fast, simple, and cost-effective viral DNA/RNA miniprep method and it is suitable for Virus DNA/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 DNA/RNA purified with TIANamp Virus DNA/RNA Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required. The purified DNA/RNA is ready for use in downstream applications such as enzymatic reactions, RT-PCR, southern blot and so on.

## Important notes

1. All protocol steps should be carried out at room temperature (15-30°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.

## Preparation of Carrier RNA solutions

- Add 310 µl RNase-Free dd$H_2O$ 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.
- Carrier RNA cannot be dissolved in Buffer GB directly. It must first be dissolved in RNase-Free dd$H_2O$ and then added to Buffer GB.
- Carrier RNA working solution: Calculate the volume of Buffer GB/ 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 0.22 ml = y ml

y ml x 28 µl/ml = z µl

n = number of samples to be processed simultaneously

y = calculated volume of Buffer GB

z = volume of Carrier RNA/RNase-Free dd$H_2O$ to add to Buffer GB

**Table 1 Volumes of Buffer GB and Carrier RNA/RNase-Free dd$H_2O$ Mix required for the Procedure**

| Number of Samples | Vol. Buffer GB (ml) | Vol. Carrier RNA/RNase-Free dd$H_2O$ (μl) |
|---|---|---|
| 1 | 0.22 | 6.2 |
| 2 | 0.44 | 12.3 |
| 3 | 0.66 | 18.5 |
| 4 | 0.88 | 24.6 |
| 5 | 1.10 | 30.8 |
| 6 | 1.32 | 37.0 |
| 7 | 1.54 | 43.1 |
| 8 | 1.76 | 49.3 |
| 9 | 1.98 | 55.4 |
| 10 | 2.20 | 61.6 |
| 11 | 2.42 | 67.8 |
| 12 | 2.64 | 73.9 |
| 13 | 2.86 | 80.1 |
| 14 | 3.08 | 86.3 |
| 15 | 3.30 | 92.4 |
| 16 | 3.52 | 98.6 |
| 17 | 3.74 | 104.7 |
| 18 | 3.96 | 110.9 |
| 19 | 4.18 | 117.0 |
| 20 | 4.40 | 123.2 |
| 21 | 4.62 | 129.4 |
| 22 | 4.84 | 135.5 |
| 23 | 5.06 | 141.7 |
| 24 | 5.28 | 147.8 |

**Note: Mix Buffer GB with Carrier RNA solution upside down. Do not vortex to avoid bubbling.**

## Protocol

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

1. Pipet 20 µl Proteinase K into a clean 1.5 ml centrifuge tube (not provided).
2. Add 200 µl of plasma/serum/ lymph into the centrifuge tube (Equilibrate the samples to room temperature.).

   **Note: If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of proteinase and sample up to a total of 220 µl.**
3. Add 200 µl Carrier RNA working solution (mixture of Buffer GB and Carrier RNA solution, please refer to table 1). Close the cap and mix by pulse-vortex for 15 s.

   **Note: In order to ensure efficient lysis, it is essential that the sample and Buffer GB are mixed thoroughly to yield a homogeneous solution.**
4. Incubate at 56°C for 15 min in a heating block. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
5. Add 250 µl of ethanol (96-100%) to the sample (Precipitates may be visible after addition of ethanol), close the cap and mix thoroughly by pulse-vortex for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15-30°C).

   **Note: Cool ethanol (96-100%) on ice before use if the room temperature is more than 25°C.**
6. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
7. Carefully transfer the lysate, including any precipitates that may have formed to the RNase-Free Spin Columns 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 spin column in the same collection tube.

   **Note: If the lysate has not completely passed through the RNase-Free Spin Columns CR2 after centrifugation, centrifuge again at higher speed until the spin column is empty.**
8. Carefully open the RNase-Free Spin Columns 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 spin column in the same collection tube.

9. Carefully open the RNase-Free Spin Columns CR2, and add 600 µl of Buffer PW **(Ensure that ethanol (96-100%) has been added before use)** without wetting the rim. Close the cap, let it stand still for 2 min and centrifuge at 8,000 rpm (~6,000 × g) for 1 min. Discard the filtrate and place the spin column in the same collection tube.
10. Repeat step 9.
11. Carefully open the RNase-Free Spin Column CR2 and add 500 µl of ethanol (96-100%) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 × g) for 1 min. Discard the filtrate.

    **Note: Ethanol carry over into the elute may cause problems in downstream applications.**
12. Place the RNase-Free Spin Column CR2 in the same collection tube. Centrifuge at full speed 12,000 rpm (~13,400 × g) 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, carefully open the cap of the spin column, incubate at room temperature for 3 min to dry the membrane completely. Add 20-150 µl of RNase-Free dd$H_2O$ to the center of the membrane. Close the cap and incubate at room temperature for 5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min.

    **Note: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl), the elution buffer should be dispensed onto the center of the membrane for complete elution of bound RNA or DNA.**