TaqGen®

Product Manual

Taqgen® Viral RNA Kit 20 preps
Cat no: MRA920

The **Taqgen® Viral RNA Kit** suitable for RNA Extraction from Fluid sample- Serum, Plasma, Urine & Nasopharyngeal Swab suspension.

For research use only.

Description

The Taqgen® Viral RNA kit, a spin column based kit, provide an easy and rapid process for the isolation for the Viral RNA. The purified RNA can be used directly in a variety of downstream applications, including PCR, RealTime PCR. The kit can readily process various fluid samples (serum, plasma, Urine, nasopharyngeal swabs suspension and viral pellet). This kit allows for the single or multiple simultaneous processing of samples.

Product Components and Storage Conditions

Product	Storage condition	Total Vol	Vol/Reaction For 200µL sample
Carrier RNA	-20 °C	150 µL	5.6 µL
Viral Lysis Buffer	RT	20 ml	560 µL
Wash Buffer WB1	RT	8 ml conc	500 µL
Wash Buffer WB2	RT	4 ml conc	500 µL
Elution Buffer	RT	20 ml	50 µL
Spin Columns	RT	20 pc	1
Collection Tube	RT	20 pc	1

Reconstitution of buffers/reagents:

Buffer/Enzyme name	Procedure
Carrier RNA (150 µg)	(Premixed for sample pk)
Wash Buffer WB1 (8 ml conc)	Add 6 ml 100% Ethanol
Wash Buffer WB2 (4 ml conc)	Add 10 ml 100% Ethanol

Storage Conditions: Store the Taqgen® Viral RNA Kit at 15°–30°C except the carrier RNA after reconstitute.

Safety Information: The Taqgen® Viral RNA kit contains guanidine hydrochloride should be considered harmful and irritants.

Caution: Handle buffer with care. Bleach reacts with guanidine hydrochloride and should not be added to any sample waste.

Before You Begin

The procedure is optimized for use with 200 µl samples, but samples up to 250µl can be used. Small samples should be adjusted to 200µl with phosphate-buffered saline (PBS) before loading, and samples with a low viral titer should be concentrated to 200µl before processing. For samples larger than 200µl, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally, but the amounts of Buffers WB1 and WB2 used in

the wash steps usually do not need to be increased. If the initial sample volume is increased, application of the lysed sample to the Spin column will require multiple loading steps.

Protocol for Viral RNA extraction from serum/plasma.

1. **Sample Preparation& viral Lysis**
   a. Add **5.6µl carrier RNA** to **200µl** of sample (serum/plasma) and mix by gentle vortexing.
   b. Add **560µl** of **viral lysis buffer**, mix thoroughly by vortexing
   c. Incubate at room temperature for 15 min.
   d. Add **560µl ethanol** (96–100%) to the sample, and mix by pulse-vortexing for 15 s.
2. **Bind RNA**
   a. Carefully transfer **700µl** of the solution to the spin column without wetting the rim. Close the cap, and centrifuge at **11,000 x *g*** for 1 min. Discard the filtrate.
   b. Carefully open the Mini column, load the remaining volume onto spin column and centrifuge the column at **11,000 x *g*** for 1 min. Discard the filtrate.
3. **Wash and dry silica membrane**
   a. Add **500µL Wash Buffer WB1**.Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   Note: Wash Buffer WB1 must be diluted with 100% Ethanol before use. Please refer Reconstitution of buffers.
   b. Add **500µL Wash Buffer WB2**.Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   Note: Wash Buffer WB2 must be diluted with 100% Ethanol before use. Please refer Reconstitution of buffers
   c. Centrifuge for **2 min** at **11,000 x *g***.
   Note: Residual ethanol is removed during this step.
4. **Elute RNA**
   a. Place the Spin Column into a 1.5mL microcentrifuge tube. Add **50µL Elution Buffer.** Incubate at room temperature for 1 min. Centrifuge **1 min** at **11,000 x *g***.

Protocol for Viral RNA extraction from nasopharyngeal swab

1. **Sample Preparation & viral Lysis**
   a. Add **5.6 µl carrier RNA** to **200µl** of a **2ml Viral transport Media** used to collect the nasopharyngeal swabs and mix by gentle vortexing.
   b. Add **560µl of viral lysis buffer**, mix thoroughly by vortexing
   c. Incubate at room temperature for 15 min.
   d. Add **560µl ethanol** (96–100%) to the sample, and mix by pulse-vortexing for 15 s.
2. **Bind RNA**
   a. Carefully transfer **700µl** of the solution to the spin column without wetting the rim. Close the cap, and centrifuge at **11,000 x *g*** for 1 min. Discard the filtrate.
   b. Carefully open the Mini column, load the remaining volume onto spin column and centrifuge the column at **11,000 x *g*** for 1 min. Discard the filtrate.
3. **Wash and dry silica membrane**
   a. Add **500µL Wash Buffer WB1**.Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   Note: Wash Buffer WB1 must be diluted with 100% Ethanol before use. Please refer Reconstitution of buffers.
   b. Add **500µL Wash Buffer WB2**.Centrifuge for **1 min** at **11,000 x *g***. Discard the filtrate.
   Note: Wash Buffer WB2 must be diluted with 100% Ethanol before use. Please refer Reconstitution of buffers.
   c. Centrifuge for **2 min** at **11,000 x *g***.
   Note: Residual ethanol is removed during this step.
4. **Elute RNA**
   a. Place the Spin Column into a 1.5mL microcentrifuge tube. Add **50µL Elution Buffer.** Incubate at room temperature for 1 min. Centrifuge **1 min** at **11,000 x *g***.
   b. Use the purified viral RNA immediately for downstream application or store at –20°C.