NeoGeneStar
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NeoGeneStar™ Viral RNA Purification Kit

For concentration and purification of Viral RNA
from saliva, nasopharyngeal or oropharyngeal swabs sample as well as serum or plasma sample

For Research Use Only.

Not for human or animal therapeutic or diagnostic use.

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NeoGeneStar™ Viral RNA Purification Kit

NeoGeneStar™ Viral RNA Purification Kit is designed for rapid purification of viral RNA and DNA from bio-fluid samples such as serum, plasma and swab samples.

Catalog No.	M2006-01 (50 preps#)	M2006-02 (200 preps#)	M2006-A32# (96 preps）	M2006-A96# （96 preps）
Lysis Buffer	30mL	120mL	Pre-packaged	Pre-packaged
Wash Buffer	15mL	60mL	Pre-packaged	Pre-packaged
Virus Beads	2×1mL	6×1mL	Pre-packaged	Pre-packaged
75% Ethanol	prepared by the user	prepared by the user	Pre-packaged	Pre-packaged
Elution buffer	10mL	20mL	Pre-packaged	Pre-packaged

#Number of reactions demonstrated is based on 200µl sample volume.

Lysis Buffer and Wash Buffer contains chaotropic salts which are irritants. Please handle with appropriate laboratory safety measures and wear gloves.

Storage and Stability

Store Virus Beads at 4~8℃ upon arrival. Freeze and violent centrifugation should be avoided. The rest of the kit can be stored at room temperature (15~25℃).

Equipment and Reagent Preparation

1. The instrument can be sterilized by UV light before use.
2. 75% Ethanol: Need to be prepared by the user.
3. Wash Buffer: Dilute with 1:1 with Absolute Ethanol.
4. Lysis Buffer and Wash Buffer must be kept away from light. Check Lysis Buffer for precipitate before use and re-dissolve at 37℃ if necessary.

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Sample Preparation

Saliva:

Add 200µl saliva to the plate for manual or automatic isolation.

Nasopharyngeal or oropharyngeal Swabs:

For swabs with preservation solution, transfer 200µl supernatant to the plate for automatic isolation. For swabs without preservation solution, add 700µl PBS or 0.9% NaCl solution to the sample, vortex and incubate for 10min, transfer 200µl supernatant to the plate for manual or automatic isolation.

Bronchoalveolar lavage and Sputum (it is recommended to inactivate virus before DNA/RNA isolation)

Add 200µl sample to the plate for manual or automatic isolation.

Whole blood:

Centrifuge at 1500×g for 10 min. Incubate on ice for 3~5min, carefully transfer 200µl supernatant to the plate for manual or automatic isolation.

Serum:

Add 200µl serum to the plate for manual or automatic isolation.

Tissue:

1. Place up to 20mg tissue in a 2ml microcentrifuge tube each containing stainless steel bead (refer to the protocol of manufacturer for suitable size).
2. Add 400µl PBS to each tube.
3. Homogenizing the tissue according to instructions of manufacturer.
4. Centrifuge at 12000rpm for 5 min. Carefully transfer 200µl supernatant to the plate for automatic isolation.

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Automation Steps for KingFisher Duo/Duo Prime

1. Take a 96-well plate, add samples and reagents to the 96-well plate according to the table below.

   Note: The total volume of each well should not exceed 900µl or it may overflow.

Table 1. Setting of 96-well plate and reagent dosage

Well	Samples /Reagents	Volume (µl)	Instructions	Notes
Column A	Samples	≤300	Users are required to add into	—
	Lysis Buffer	600	Users are required to add into if the reagent has not been pre-packaged	—
Column B	Wash Buffer	600	Users are required to add into if the reagent has not been pre-packaged	Ethanol must be added prior to use
Column C	75% Ethanol	600	Users are required to add into if the reagent has not been pre-packaged	—
	Virus Beads	30	Users are required to add into if the reagent has not been pre-packaged	—
Column D	75% Ethanol	600	Users are required to add into if the reagent has not been pre-packaged	—
Elution Strip	Elution Buffer	60	Users are required to add into if the reagent has not been pre-packaged	—
Column H	12 DP tip comb			

Note: For M2006-01 and M2006-02, you are required to add reagent and Virus Beads into

plate, we recommend you use multichannel pipettor.

2. Start the instrument and set the procedure according to Table 2.
3. Place a new clean magnetic rod's tip in the instrument (Be sure to replace the magnetic rod's tip with a new one, or it will cause cross-contamination of the sample). Place the 96-well plate containing the sample and reagent into the instrument, corresponding to the magnetic rod.

   Note: Remember to place the magnetic rod's tip, or the reagent will erode the magnetic bar, affecting the performance of the instrument and subsequent experiments.
4. Execute procedures.
5. Collect RNA/DNA: Remove the 96-well plate and magnetic rod's tip. Transfer the cleared supernatant into a new tube and store at -20℃ or -80℃.

Table 2. Program Setting of KingFisher Duo/Duo Prime

Steps	Well	Release Beads		Mixing/Heating Parameters			Collect Beads	
		Time	Speed	Time	Speed	Heating Temperature	Time/ Cycle	Cycle
Pick up Tip	Column H	--						
Collect	Column C	--	--	30sec	Fast	--	10s/Cycle	3 Cycles
Binding	Column A	--	--	7min	Fast	65℃	10s/Cycle	3 Cycles
Wash 1	Column B	--	--	30sec	Fast	--	10s/Cycle	3 Cycles
Wash 2	Column C	--	--	30sec	Fast	--	10s/Cycle	3 Cycles
Wash 3	Column D	--	--	30sec	Fast	--	10s/Cycle	3 Cycles
Dry	Column D	Outside well/tube 2min						
Elution	Elution Strip	--	-	2min	Medium	65℃	20s/ Cycle	3 Cycles
Leave Tip	Column D	--						

Automation Steps for KingFisher Flex

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1. Take 5 96-well plates, add samples and reagents to 96-well plates according to Table 3.

   Note: The total volume of each well should not exceed 900µl or it may overflow.

Table 3. Setting of 96-well plate and reagent dosage

Step	Plate Position	Samples /Reagents	Volume (µl)	Instructions	Notes
Binding	1	Samples	≤300	Users are required to add into	—
		Lysis Buffer	600	Users are required to add into if the reagent has not been pre-packaged	Check Lysis Buffer for precipitate before use and re-dissolve at 37℃ if necessary.
Wash 1	2	Wash Buffer	600	Users are required to add into if the reagent has not been pre-packaged	Dilute 1:1 with Absolute Ethanol
Wash 2	3	75% Ethanol	600	Users are required to add into if the reagent has not been pre-packaged	—
		Virus Beads	30	Users are required to add into if the reagent has not been pre-packaged	—
Wash 3	4	75% Ethanol	600	Users are required to add into if the reagent has not been pre-packaged	—
		Magnetic rod's tip	—	—	Put the 96-magnetic rod's tip into this plate.
Elution	5	Elution Buffer	60	Users are required to add into if the reagent has not been pre-packaged	—

Note: For M2006-01 and M2006-02, you are required to add reagent and Virus Beads into plate, we recommend you use multichannel pipettor.

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2. Start the instrument, place the 6 plates and the magnetic rod's tip on the corresponding position in the instrument.
3. Execute procedures (Table 4).
4. Collect RNA/DNA: Remove the 96-well plates and magnetic rod's tip. Transfer the cleared supernatant of elution plate into new tubes and store at -20℃ or -80℃ .

Table 4. Program Setting of KingFisher Flex

Steps	Position	Release Beads		Mixing/Heating Parameters			Collect Beads	
		Time	Speed	Time	Speed	Heating Temperature	Time/ Cycle	Cycle
Pick up Tip	4	--						
Collect	3	--	--	30 sec	Fast	--	10s/ Cycle	3 Cycles
Binding	1	--	--	7 min	Fast	65℃	10s/ Cycle	3 Cycles
Wash 1	2	--	--	30 sec	Fast	--	10s/ Cycle	3 Cycles
Wash 2	3	--	--	30 sec	Fast	--	10s/ Cycle	3 Cycles
Wash 3	4	--	--	30 sec	Fast	--	10s/ Cycle	3 Cycles
Dry	4	Outside well/tube, 2min						
Elution	5	--	--	2 min	Medium	65℃	20s/ Cycle	3 Cycles
Leave Tip	4	--						

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Manual steps for virus RNA / DNA isolation

1. Add 200µl sample into a 2ml microcentrifuge tube.
2. Add 600µl Lysis Buffer.

   Note: If the sample volume is less than 200µl , add the appropriate volume of 0.9% NaCl solution to bring the volume of sample up to a total of 200µl. If the sample volume is more than 200µl, increase the Lysis Buffer volume at Lysis Buffer : Sample equals 3:1.
3. Fully resuspend Virus Beads by vertexing for 1 minute. Add 30µl Virus Beads, and completely resuspend the magnetic particles by vertexing or pipetting up and down for 10 times. Incubate at 65℃ for 7~10 minutes. Mix the tube every 2~3 minutes during incubation.

   Note: Virus Beads tend to settle to the bottom, it is very important to resuspend the beads thoroughly before use to ensure a homogeneous mix of this reagent is transferred into each tube to avoid the difference between tubes.
4. Place the tube onto a magnetic stand for 1 minute and aspirate the supernatant with pipette carefully without aspirating the magnetic beads.
5. Remove the tube from the magnetic stand and add 600µl Wash Buffer into the tube. Completely resuspend the magnetic beads by vertexing and mix for 1 minute.

   Note: Ethanol must be added prior to use into the bottle labeled Wash Buffer .
6. Place the tube onto a magnetic stand for 1 minute or until the solution clears. Aspirate the cleared supernatant with a pipette carefully without aspirating the magnetic beads and then discard the supernatant.
7. Remove the tube from the magnetic stand and add 600µl 75% Ethanol to the tube. Completely resuspend the magnetic beads by vertexing and mix for 1 minute.
8. Place the tube onto a magnetic stand for 1 minute or until the solution clears. Aspirate the cleared supernatant with a pipette carefully without aspirating the magnetic beads and then discard the supernatant.
9. Wash the magnetic beads again by repeating step 7 and 8.
10. Remove any trace of liquid with pipette tips. Air-dry the magnetic beads by placing the tube at room temperature for 10-20 minutes.

    Note: Do not vacuum dry, and excessive drying can lower the recovery rate.
11. Add at least 60µl Elution Buffer and resuspend the magnetic beads by pipetting up and down for 10 times or vertexing. Incubate at 65℃for 2~5 minutes.
12. Place the tube onto a magnetic stand to magnetize the beads. The solution should be cleared after all magnetic beads are completely pelleted.
13. Transfer the cleared supernatant into a new tube.