SAFENSURE™ Virus DNA Sample Preparation Kit (Magnetic Beads)

User Manual V1.0

Catalog Number: OPA-E103

Assay Tests: 50 Preps

IMPORTANT: Please carefully read this user guide before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.

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Product Information

The SAFENSURE™ Virus DNA Sample Preparation Kit is designed for the efficient extraction of viral DNA from biological products, including recombinant proteins, monoclonal antibodies, fusion proteins, vaccines, recombinant enzymes, and cell banks. The kit provides a convenient and robust integrated solution for viral DNA extraction, supporting sample preparation for downstream detection of adventitious viruses by qPCR.

Prior to virus DNA detection, DNA extraction from samples is required, and extraction using this kit is recommended. This kit utilizes magnetic bead-based nucleic acid extraction technology. Under optimized buffer conditions, magnetic beads act as solid-phase carriers that selectively bind DNA molecules. The magnetic beads are rapidly separated from the liquid phase using an external magnetic field, while impurities such as proteins and salts are removed through a series of washing steps. Purified DNA is subsequently eluted from the magnetic beads using an elution buffer and is directly compatible with downstream qPCR assays. It is suitable for both manual processing and high-throughput automated DNA extraction when used in conjunction with nucleic acid extraction instruments. This manual describes protocols for manual extraction and automated workflow compatible with the ACRO OPE-32S Automated Nucleic Acid Extraction System.

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Important Notes

1. Application Scope: It is used for in vitro research only, and must not be used for clinical diagnosis.
2. The kit shall be stored in accordance with the storage conditions specified in the instructions and used within the validity period.
3. Operational Specifications: Read this manual carefully before use, and operations shall be performed strictly in accordance with the manual.
4. Laboratory Zoning: It is recommended to perform PCR reaction setup, sample extraction and DNA template addition, and qPCR amplification in physical separated areas. Ensure that each area is kept clean to minimize the risk of cross-contamination.
5. Anti-Contamination Measures:
   - Clean and disinfect the experimental area before and after each operation, including the workbenches, desktops, equipment surfaces, pipettes. Use a nucleic acid decontamination solution when necessary.
   - Wear disposable powder-free gloves, mask, and a hair cover during operation.
   - Use sterile, nuclease-free, and low-adsorption consumables such as microcentrifuge tubes and filter pipette tips.
   - Change pipette tips between samples to prevent cross-contamination.
   - Keep tubes closed whenever possible and minimize the duration of exposure to the environment.
6. Liability Clause: The company guarantees the quality and performance of the kit itself. Please note that sample loss during experimental procedures falls outside the scope of our quality. To ensure the success of your experiment, we recommend evaluating sample requirements in advance and allocating appropriate backup samples accordingly.

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Contents

The kit can be used for 50 preps of viral DNA extraction from test samples.

Contents	Amount
Binding Buffer L	35 mL
Proteinase K	4 mL
MagBeads Suspension	1.4 mL
CR Powder	310 μg
Wash Buffer Ⅰ	38 mL
Wash Buffer Ⅱ	18 mL
Elution Buffer	5 mL
Sample Dilution Buffer	5 mL

The unopened reagents can be stored stably for 18 months from the date of manufacture when kept at a storage temperature between 10℃ and 30℃.

In an environment with a low temperature (< 20℃) , precipitates may form in Binding Buffer L and Wash Buffer Ⅰ . Place the reagent bottles in a water bath at 37℃-50℃ for about 10 minutes until the precipitates are completely dissolved before use.

NOTE: Once opened, store Proteinase K and MagBeads Suspension at 2-8°C.

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List of Abbreviations

Abbreviations	Full Name
NEC	Negative Extraction Control
PEC	Positive Extraction Control
PC	Positive Control
IC	Internal Control

PART A: Manual DNA Extraction

Required Materials Not Supplied

Category	Item	Experimental Recommendation
Equipment	Magnetic stand	Surface magnetic field > 5000 gauss
	Block heater	Compatible with 2 mL centrifuge tubes
	Vortex	2500-3000 rpm
	Mini centrifuge	3000-6000 rpm
	Pipettors	1000 µL, 200 µL, 100 µL, 20 µL, 10 µL
Reagent	Ethanol	GR, 99.8%
Consumables	Disposable gloves	Dust-free, sterile
	Pipette tips	Nuclease-Free, DNA-Free, with filter.
	1.5 mL/2.0 mL Microcentrifuge tubes	Nuclease-Free, DNA-Free, low-DNA binding

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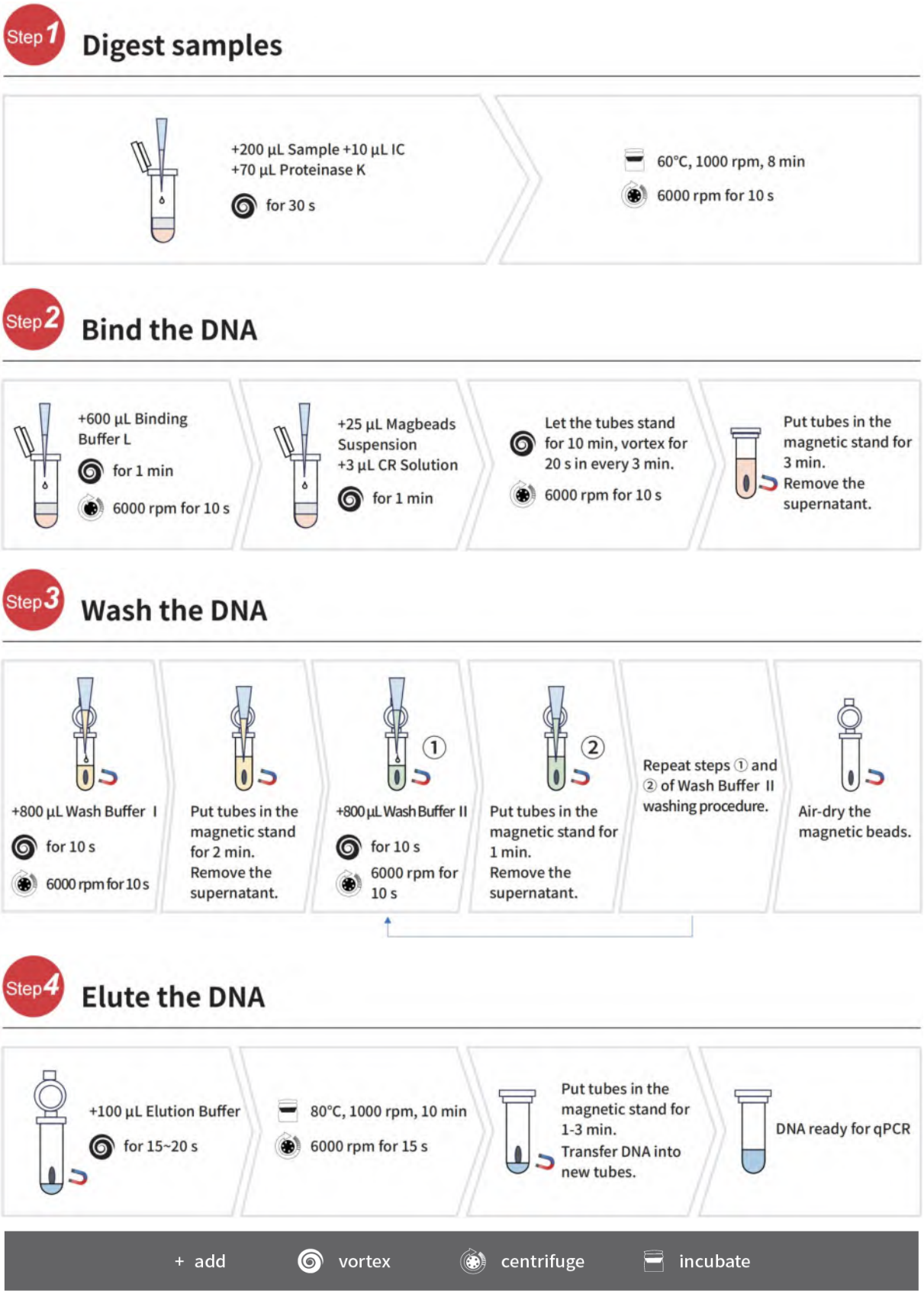
Workflow for Manual DNA Extraction



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Prepare Reagents and Samples for Manual DNA Extraction

1. Prepare Reagents Before First Use of the Kit.

1.1 For newly opened Wash Buffer Ⅰ, add ethanol according to the volume indicated on the label, mix thoroughly before use.

1.2 For newly opened Wash Buffer Ⅱ, add ethanol according to the volume indicated on the label, mix thoroughly before use.

1.3 Incubate the MagBeads Suspension at room temperature for 30 minutes in advance. Before being used, the magnetic beads have to be vigorous vortexed and mixed.

NOTE: The MagBeads Suspension should be mixed for at least 1 minute until evenly dispersed. Invert the tube several times and visually confirm the bead status. No bead should adhere to the bottom of the tube, and the suspension should be uniformly dispersed with a consistent color and no dark aggregates or clumps.

1.4 Preparation of CR Solution: Briefly centrifuge the CR Powder tube, then add 310 µL of DNase/RNase-Free Water (provided with our qPCR detection kit) to the tube, and vortex thoroughly.

NOTE: The CR Solution should be stored at -20℃. Aliquot into small volumes to minimize freeze-thaw cycles, ensuring no more than three cycles.

2. Prepare Samples

2.1 Sample Pretreatment

The recommended sample input volume for extraction is 200 µL. Different sample types should be pre-treated according to the instructions below before extraction.

2.1.1 **Small-volume samples (without cells):** If the sample volume is 200 µL, perform DNA extraction directly. If the sample volume is less than 200 µL, made up the volume to 200 µL with Sample Dilution Buffer before extraction.

2.1.2 **Cell suspension:**

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- When the cell concentration is no more than 10^6 cells/mL, mix the sample thoroughly and take 200 µL of the sample for extraction.
- When the cell concentration is greater than 10^6 cells/mL, first take 220 µL of the cell sample and centrifuge at 100 ×*g* for 5 minutes. After centrifugation, transfer 200 µL of the supernatant to a new centrifuge tube – just be careful not to pipette the pellet.

2.1.3 **Non-cellular sample:** When the sample is in powder form, please dissolve the sample with the Sample Dilution Buffer and dilute it to 1 mg/mL-100 mg/mL, then take 200 µL of the sample for extraction.

2.2 Prepare the Test Samples

After sample pretreatment, add 200 µL of the sample to a 2.0 mL microcentrifuge tube. Then add **10 µL of IC,** close the tube securely and label it as "Test Sample".

2.3 Prepare the NEC

For each extraction run, you'll need to set up a negative extraction control. Add 200 µL of Sample Dilution Buffer and **10 µL of IC** into a 2.0 mL microcentrifuge tube. Close the tube securely and label it as "**NEC**".

2.4 Prepare the PEC

Add 100 µL of **PC**, 100 µL of Sample Dilution Buffer, and **10 µL of IC** into a 2.0 mL microcentrifuge tube, then label it as "**PEC**".

NOTE: The PC and IC are included in the qPCR detection kit.

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Protocol for Manual DNA Extraction

1. Digest Samples

1.1 Prepare the "**Test Samples**", "**NEC**" and "**PEC**" according to section "2. Prepare Samples" on page 6.

1.2 Add **70 µL of Proteinase K** to each tube, vortex for 30 seconds and briefly centrifuge.

1.3 Incubate the tubes at 60 °C for 8 minutes on a block heater, with vortexing at 1000 rpm (heating lid is recommended). After incubation, centrifuge briefly and place the tube on a rack to cool to room temperature.

NOTE: Briefly centrifuge: It is recommended to centrifuge at 3000-6000 rpm for 5-10 seconds. It is applicable to all of briefly centrifuge mentioned in this instruction manual.

2. Bind the DNA

2.1 Add **600 µL of Binding Buffer L** to each tube, then close the cap and invert several times to mix, vortex for 1 minute and briefly centrifuge.

2.2 Add **25 µL of MagBeads Suspension** and **3 µL of CR Solution** to each tube, close the cap and invert several times to mix. Then vortex for 1 minute. Let the tubes stand for 10 minutes, vortexing for 20 seconds every 3 minutes in between.

NOTE: (1) To ensure accurate dispensing of magnetic beads, vortex the MagBeads Suspension for at least 1 minute before use.

(2) After adding 3-4 samples, please vortex the suspension again for about 30 seconds before continuing to add samples.

(3) To ensure uniformity, it is recommended to gently pipette the suspension up and down twice before each aspiration.

2.3 Vortex all tubes for 1 minute. Incubate the tubes at room temperature for 10 minutes. During incubation, vortex for 20 seconds every 3 minutes.

2.4 Briefly centrifuge the tubes, then place them on a magnetic stand with the beads

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collected against the magnet. Let the tubes stand for 3 minutes or until the solution is clear.

2.5 Without disturbing the magnetic beads, carefully remove and discard the supernatant using a pipette or by aspiration.

3. Wash the DNA

3.1 Add **800 µL of Wash Buffer I** to each tube (just double-check that ethanol has been added before use). Vortex for 10 seconds, then centrifuge briefly.

3.2 Put the tubes on a magnetic stand and leave it for 2 minutes – wait for the solution to clear and all beads to be captured.

3.3 Without disturbing the magnetic beads, carefully remove and discard the supernatant using a pipette or by aspiration.

3.4 Add **800 µL of Wash Buffer II** (check that ethanol has been added beforehand), then vortex for 10 seconds.

3.5 Briefly centrifuge the tubes, then put the tubes on a magnetic stand, let the tubes stand for 1 minute or until the solution becomes clear.

3.6 Without disturbing the magnetic beads, carefully remove and discard the supernatant using a pipette or by aspiration.

3.7 Repeat the steps 3.4-3.6.

3.8 Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 30 seconds or until the solution is clear.

3.9 Use a 10 µL pipette to remove any solution from the bottom of the tube.

3.10 With the tube lid open, air-dry the magnetic beads in the magnetic stand for no more than 2-3 minutes at room temperature.

NOTE: (1) Residual ethanol may interfere with the subsequent PCR reaction, please ensure that the ethanol is completely volatilized. At the same time, avoid over-drying the magnetic beads, which could make nucleic acid elution difficult.

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(2) Observe the magnetic beads under bright light, add the Elution Buffer immediately when the surface of the magnetic beads appears dull.

(3) If the surface of the magnetic beads is yellowish-brown, it indicates excessive drying, which will affect the recovery rate of nucleic acid.

4. Elute the DNA

4.1 Add **100 µL of Elution Buffer** to each tube, then resuspend the beads by vortexing for 15-20 seconds or pipetting up and down until suspension is fully homogenized.

4.2 Incubate the tubes at 80°C for 10 minutes on a block heater, with vortexing at 1000 rpm.

4.3 Briefly centrifuge the tubes for 15 seconds, then place the tubes on a magnetic stand, let the tubes stand for 1-3 minutes or until the solution is clear.

4.4 Use a 100 µL pipette to transfer the liquid phase to a new 1.5 mL microcentrifuge tube. (Do not disturb the magnetic beads).

NOTE: If the DNA sample is to be tested by qPCR within 24 hours, it can be temporarily stored at 2–8°C. For long-term storage, it should be kept at –20°C, and the test should be completed within 7 days.

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PART B: Automated DNA Extraction: Application to ACRO OPE-32S

Required Materials Not Supplied with the ACRO OPE-32S

Category	Item	Experimental Recommendation
Equipment	Automated Nucleic Acid Extraction System	ACRO OPE-32S
	Vortex	2500-3000 rpm
	Mini centrifuge	3000-6000 rpm
	Pipettors	1000 µL, 200 µL, 100 µL, 20 µL, 10 µL
Reagents	Ethanol	GR, 99.8%
Consumables	Disposable gloves	Dust-free, sterile
	Pipette tips	Nuclease-Free, DNA-Free, with filter
	1.5 mL/2.0 mL microcentrifuge tubes	Nuclease-Free, DNA-Free, low-DNA binding
	96 V-Bottom Deep-well Plate	Cat. No.: OPA-R013
	8-Strip V-Bottom Tip Comb	Cat. No.: OPA-R014

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Workflow for Automated DNA Extraction Using the ACRO OPE-32S

*Binding Buffer: Binding Buffer L 600µL+CR Solution 3 µL;
*IC: Internal Control DNA, add 10 µL IC to each sample (include test samples, negative extraction control and positive extraction control) need to be extracted.

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Prepare Reagents and Samples for Automated DNA Extraction Using the ACRO OPE-32S

1. Prepare Reagents Before First Use of the Kit

1.1 Please refer to manual DNA extraction, point 1 on page 6.

2. Prepare Samples

2.1 Please refer to manual DNA extraction, point 2 on page 6.

Protocol for DNA Extraction by ACRO Automated Extraction System (OPE-32S)

The following steps apply for ACRO Automated Nucleic Acid Extraction System (OPE-32S).

NOTE: When using automated nucleic acid extraction equipment from third-party manufacturers, refer to this instruction manual for general procedures. Adjust the steps as needed according to the specific instrument configuration.

1. Instrument Preparation

1.1 Before using the instrument, wipe down the processing chamber with **75% ethanol**.

1.2 Close the chamber door, activate the **UV lamp program**, and irradiate the chamber with UV light for **15 minutes**.

2. Reagents Dispensing

2.1 Following the 96-Well Deep Plate Reagent Dispensing Schematic, add the required reagents to the designated wells of the deep-well plate.

2.2 Pipette **70 µL of Proteinase K** into column 1 or 7 of the deep-well plate.

2.3 Dispense **800 µL of Wash Buffer I** into column 2 or 8 of the deep-well plate.

2.4 Add **800 µL of Wash Buffer II** to column 3 or 9 of the deep-well plate.

2.5 Transfer **800 µL of Wash Buffer II** to column 4 or 10 of the deep-well plate.

2.6 Load **100 µL of Elution Buffer** into column 5 or 11 of the deep-well plate.

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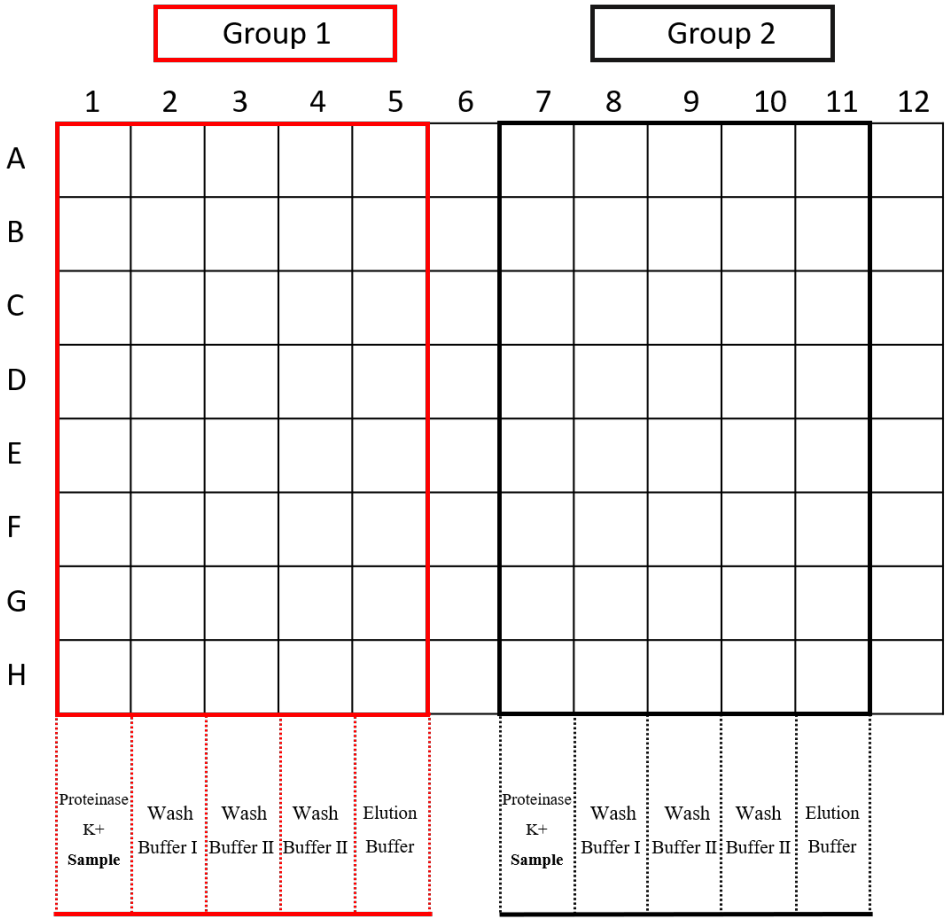

96-Well Deep Plate Reagent Dispensing Schematic

3. Sample Digestion

3.1 Transfer 210 µL of prepared sample (test sample + IC) into Column 1 or 7 of the 96-well deep plate.

3.2 Place the loaded plate into the Deep Well Plate Station of the instrument. Insert the 8-strip V-bottom tip comb into the corresponding slot until fully seated. Close the chamber door, and run program **OPA_E103_A**. This program is preloaded in the ACRO OPE-32S Automated Nucleic Acid Extraction System.

NOTE: Ensure proper placement of the deep-well plate. Verify the 8-strip V-bottom tip combs are properly inserted and fully seated.

3.3 After program completion, click "Complete" on the screen. First remove the 8-strip V-bottom tip combs, then take out the deep-well plate.

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4. Samples Extraction

4.1 Prepare the Binding Buffer according to the number of samples to be processed. To count for potential loss during preparation and pipetting, it is recommended to include one additional sample volume in the calculation. The total preparation volume should be **N**=**n**+1, where **n** is the number of samples. Prepare the Binding Buffer according to the following table:

Reagents	Volume/Sample	Binding Buffer Volume
Binding Buffer L	600 µL	600 µL×**N**
CR Solution	3 µL	3 µL×**N**
Total	603 µL	603 µL×**N**

4.2 After sample digestion, first add 603 µL of Binding Buffer to Column 1 and 7, then add 25 µL of MagBeads Suspension.

NOTE: The Binding Buffer must be thoroughly mixed before dispensing. The magnetic beads must be vortexed completely before addition.

4.3 Place the loaded plate back to the instrument and insert new 8-strip V-bottom tip combs. Close the chamber door and run program **OPA_E103_B**. This program is preloaded in the ACRO Automated Nucleic Acid Extraction System (OPE-32S).

4.4 After program completion, click "Complete" on the screen, remove the 8-strip V-bottom tip combs, and take out the deep-well plate. Immediately transfer Elution Buffer from **Column 5 or 11** to new 1.5 mL microcentrifuge tubes or PCR tubes for storage. The eluted DNA can be used for subsequent qPCR detection.

NOTE: (1) For qPCR detection within **24 hours**, DNA samples could be stored temporarily at **2-8°C.**

(2) For long-term storage, keep at **-20°C** and complete testing within **7 days.**

(3) Multichannel pipettes are recommended for faster operation during transfer.

4.5 After experiment completion, wipe the instrument processing chamber with 75%

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ethanol. Close the chamber door, activate the **UV lamp program**, and irradiate the chamber with UV light for **30 minutes.**

NOTE: Maintain an interval of at least 30 minutes between experiments to prevent cross-contamination.

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Appendixes

Appendix A: FAQs

Common Questions	Possible Reasons & Actions
Do precipitates appearing in Binding Buffer L affect extraction efficiency?	Precipitates appearing in Binding Buffer L may be caused by low temperature. Please dissolve the precipitates according to the instruction of part "Contents" on page 3. It has no effect on the extraction efficiency after the precipitates are completely dissolved. The laboratory temperature should be kept at 20℃ to 30℃ day and night, and the temperature should not be lower than 20℃ to avoid precipitates appearing in Binding Buffer L.
How long can the eluted DNA be stored without affecting the testing results?	It is recommended to finish qPCR testing on the same day as extraction or within 24 hours. The DNA can be temporarily stored at 2°C to 8°C. For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days. Also, it should be frozen and thawed only once.
Does the kit stored at room temperature affect the extraction efficiency?	The kit has been verified and can be stored at room temperature (10-30°C) for one year. For optimal long-term stability, the MagBeads Suspension and Proteinase K are recommended to be stored at 2℃ to 8℃ after the kit is opened.
What could be done if the extraction efficiency of high-protein samples is poor?	For samples with high protein concentration, you can try to extend the digestion time of the samples with Proteinase K from 8 minutes to 30 minutes.

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Can samples with high cell concentration (10^7 cells/mL) be extracted directly for virus detection?	It is not recommended for extraction directly. An excessive amount of cellular genomic DNA and/or RNA may be extracted during extraction owing to high concentration cells, which will lead to the inhibition of qPCR and affect the detection of low-content targets. Please pre-treat the samples according to the instruction of part "Prepare Samples: 2.1.2 Cell suspension" on page 7.

Appendix B: Consumables recommendation

Consumables	Catalog Number	Manufacturer
96 V-Bottom Deep-well plate	OPA-R013	ACRO
8-Strip V-Bottom Tip Comb	OPA-R014	ACRO
2.0 mL Low DNA-Binding Microcentrifuge Tubes	72.695.700	SARSTEDT
1.5 mL Low DNA-Binding Microcentrifuge Tubes	72.706.700	SARSTEDT

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