One-Step Swab & Saliva Viral RNA/DNA Purification Kit

Swab/Saliva virus RNA/DNA purification is of paramount importance in the field of diagnostics, particularly in identifying viral infections like COVID-19. This procedure plays a crucial role in obtaining high-quality RNA/DNA samples from patient swabs/Saliva, enabling accurate and reliable virus detection. Due to the very low amount of virus present in the samples, the virus RNA/DNA purification techniques must be a robust and reliable extraction system to deliver pure and high-quality nucleic acids for sensitive downstream applications. Currently available virus RNA/DNA purification kits present challenges such as low RNA/DNA yield, time-consuming, laborious, or contamination with PCR inhibitors. They require expensive laboratory settings and well-trained personnel. To overcome these hurdles, Bioclone offers a novel extraction-free nucleic acid purification technique for trace amounts of viral samples, a robust alternative for viral nucleic acid extraction that guarantees a stable and scalable high-throughput extraction workflow at a low cost from the sample.

BcMag™ One-Step Swab/Saliva Viral RNA-DNA Purification Kit uses novel negative selection chromatography magnetic beads to quickly capture impurities such as PCR inhibitors from cell lysate, leaving the DNA /RNA untouched. Unlike standard tedious bind-wash-elute protocol, this convenient single-step and the extraction-free procedure does not contain traces of organic solvents, chaotropic salts, or EDTA, reducing the risk of DNA/RNA loss and carryover of extraction buffers, and recovers almost 100% DNA/RNA. The purification kit provides a fast and simple method for DNA/RNA purification with only one tube, no liquid transfer, and no requirement for carrier RNA. Hundreds of trace samples such as saliva and nasal-pharyngeal swabs in saline solution, nasal swabs, buccal swabs & sputum/saliva swabs can be processed in less than 15 minutes without using expensive types of equipment. The purified nucleic acid is ready for downstream applications: PCR, RT-PCR, RT-qPCR, LAMP, RT-LAMP.

Workflow (Fig.1)

1. Add cell lysis buffer to the sample.
2. Incubate at room temperature for 5 minutes.
3. Add functional magnetic beads to the sample.
4. Mix the samples with the magnetic beads.
5. Mix by pipetting or vortexing to capture the impurity.
6. Magnetic capture the beads and aspirate the supernatant containing the pure ready-to-use DNA/RNA

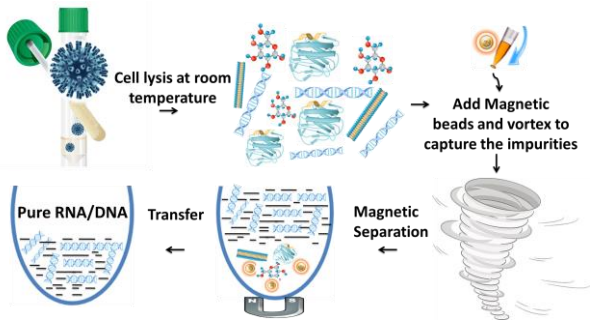

Fig.1

Features and Advantages

1. **10-Minute Protocol:** Our kit streamlines the extraction process, enabling you to obtain high-quality viral RNA/DNA in just 10 minutes, saving you valuable time.
2. **High Throughput:** Designed for high-throughput applications, this kit allows you to process hundreds of samples simultaneously, making it ideal for large-scale testing or research projects. Compatible with many different automated liquid handling systems.
3. **Room Temperature Lysis:** Our kit features a 5-minute room temperature lysis step, ensuring the stability of your samples and simplifying the workflow.

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4. **One-Step, One-Tube:** With our one-step, one-tube solution, you can directly extract viral RNA/DNA from swab and saliva samples in a single tube, reducing the risk of contamination and minimizing handling steps.
5. **PCR Inhibitor Free:** Our innovative beads efficiently eliminate inhibitors (Fig.2), including polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, heparin, detergents, denim dyes, divalent cations like Ca^{2+} and Mg^{2+}. This ensures reliable and accurate results.
6. **High-Quality Nucleic Acid:** Trust in the quality of your extracted nucleic acids. Our kit consistently delivers high-quality RNA/DNA, meeting the stringent requirements of your research or diagnostic needs.

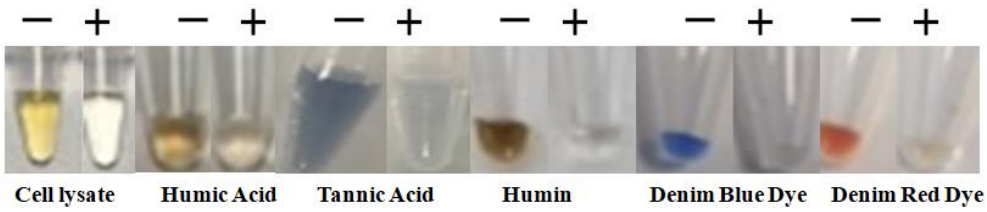

Fig.2

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	50 preps, Cat # AR-101	100 preps, Cat # AR-102
BcMag™ L27 Beads	4°C	2.5 ml	5.0 ml
10x Lysis Buffer	4°C	0.6 ml	1.2 ml
DTT	-20°C	15.4 mg	30.8 mg

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA Yield: Varies (depends on sample size and type)
- DNA Size: Varies (depends on the quality of starting material
- Since there is no concentration step in the protocol, the concentration of the nucleic acid depends on the quality and quantity of the sample used.
- Quantification of nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green.
- OD_{260} methods such as Nanodrop and UV-spectrophotometry are not-suitable.
- For long-term storage, store the extracted nucleic acids at -20°C.

Materials Required by the User

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag™ Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag™ 96-well Plate Magnetic Rack.	• BcMa™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-06)

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Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the Time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	

A. Sample preparation

Handling Samples (Table1)
Follow these general guidelines when handling forensic samples:

- Only synthetic fiber swabs and rigid plastic rods should be used. Cotton fiber contains substances that inactivate the virus and can inhibit the RT-PCR test leading to false negatives.
- We recommend that swabs be collected in Molecular diagnosis compatible VTM (Viral Transfer Medium). Incompatible VTM may lead to lower or no nucleic acid recovery or Ct value delays.

Table 1.

Sample	Example sample input
Buccal swab	General Swab Collection Instructions • Apply appropriate but significant pressure to the body site to be tested while rotating the swab. • This ensures that adequate cells (viruses and chlamydial species are intracellular) are obtained. • Agitate swab briskly in vial containing viral transport media (100-150µl). • Hold the end of the swab shaft; bend it at 180-degree angle to break at the marked breakpoint. Place tip in vial and secure the lid tightly.
mouthwash	1. Add 500µl-1000µl mouthwash into a new 1.5 ml centrifuge tube. 2. Centrifuge at 14,000 rpm for 5 minutes to pellet the cell. 3. Aspirate all the liquids. 4. Add 1 ml ultrapure water to the tube, Vortex, and centrifuge at 14,000 rpm for 5 minutes to pellet the cell. 5. Remove all the liquids. 6. Resuspend the cell pellet with 100µl ultrapure water. Note: Food, drinks, smoking, or chewing gum should be restricted up to 30 min to 1 h before giving your saliva sample.

B. Premix Beads solution Preparation

IMPORTANT!

1. Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
2. Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.
3. DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 1M in ultrapure water. For example, 15.4 mg dissolved in 100µl ultrapure water. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.
4. Dilute DTT to a concentration of 10 mM from stock with ultrapure water and use it immediately. Discard unused DTT solution.

C. Isolation procedure

IMPORTANT!

- Pipette up and down premix beads solution in a reagent reservoir until the solution is homogeneous before dispensing.

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- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.)

1. Pipette 100 µl of the sample into a suitable container or microcentrifuge tube.
2. Add 10x lysis buffer to the sample at a 1:10 ratio. This means adding 10 µl of the 10x lysis buffer for every 100 µl of the sample.
3. Prepare a 10 mM DTT solution by diluting the DTT reagent in ultrapure water.
4. Pipette 3 µl of the 10 mM DTT solution for every 100 µl of the diluted sample. For example, if you have 200 µl of the diluted sample, add 6 µl of the DTT solution.
5. Mix the sample gently to ensure thorough mixing.
6. Incubate at room temperature for 5 minutes.
7. Add 50 µl BcMag™ L27 Beads
8. Mix the sample with beads by slowly pipetting up and down 20-25 times or Vortex the sample at 2000 rpm for 5 minutes (see picture).

9. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
10. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications. Using 1-5 µl in a 25µl RT-PCR or qPCR.

D. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA/RNA Recovery	Poor starting sample material.	• Use better-quality samples. • Add more samples
Ct value delays	Too many PCR inhibitors in the sample.	1. Add 25-50 µL BcMag™ L27 Beads to the extract solution and mix by slowly pipetting up and down 20-25 times or Vortex the sample at 2000 rpm for 5 minutes. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear. 2. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. Using 1-5 ul in a 25µl RT-PCR or qPCR. The sample is ready for downstream applications.
	Recovery DNA is so low.	• Use a better-quality sample. • Add more samples.

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Tube Swab/Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101

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DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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