



Clinical Sample Processing Guide

Version 1.1





Clinical Sample Processing Guide

Introduction

This *Clinical Sample Processing Guide* is aimed at helping Bioneer *AccuPrep*[®], *ExiPrep*[™] and *ExiStation*[™] product users understand the steps involved in preparing clinical samples for kit input. The protocols contained herein have been developed and proven in the field to maximize nucleic acid extraction efficiency and purity. We recommend that users follow the processing protocols within this guide for best results. Adherence to the protocols within this guide has shown to yield dramatic improvement in the quality of data obtained from the end-use of extracted nucleic acids (especially molecular diagnostics).

We are providing this guide as a convenience to users of our nucleic acid extraction products. We hope that this guide will enable our customers to experience the full performance our products have to offer.

Safety Warnings and Precautions

Please obtain a copy of Material Safety Data Sheets (MSDS) or products in use from BIONEER's Customer Service Center if necessary. Please follow all regulations with regards to handling clinical samples, reagents, media etc.

Clinical samples must be handled by people trained in molecular biology or clinical experimentation. Please read through this guide before performing any steps. Also, ensure that reagents that may be used while following this guide are present and good.

Always wear protection when handling clinical samples, and make sure that there is no direct contact with your body. If any direct contact occurs, treat promptly and properly according to the hospital/center's regulations. Always use sterilized consumables including tips and tubes to prevent fouling or contamination of samples.



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1 Processing Sputum

1.1 Sample amount: > 1 mL

1.2 Storage conditions and period: Refrigerated (7 days), Frozen (> 7 days)

1.3 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, 10N NaOH, 1X PBS (pH 6.8~7.2)

1.4 Method

① Pour 1/10 v/v 10 N NaOH in the sputum sample container and vortex hard for 3 minutes.

② Incubate at room temperature (25°C) for 15 minutes.

Note) If the sample is excessively viscous, load up to twice the volume of NaOH or extend the incubation period up to 30 minutes. In case of that the sample is still viscous in spite of doubled volume of NaOH, transfer the sample to a conical tube and add equivalent amount of PBS or DEPC water to the sample then vortex the tube.

③ Vortex the sample hard for 1 minute. Transfer 1.5 mL to a sterilized 1.5 mL microcentrifuge tube.

④ Centrifuge at 9,000 rpm for 5 minutes and completely remove the supernatant.

Note) If a white layer forms above the cell pellet, remove the layer with the supernatant and leave the cell pellet.

⑤ Add 1 mL of 1x PBS into 1.5 mL microcentrifuge tube and vortex for 30 seconds to wash the cell pellet. Centrifuge at 9,000 rpm for 5 minutes and completely remove the supernatant. Repeat this washing step 2~3 times.

Note) If a white layer forms above the cell pellet, remove the layer with the supernatant and leave the cell pellet.

⑥ Transfer 400 µL of Resuspension Buffer, 1x PBS or normal saline and completely dissociate the cell pellet using a pipette.

⑦ Briefly spin-down at 6,000 rpm for 5 seconds. The sample is now ready to be used.



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2 Processing Fluids

2.1 Sample type: Particle-free body fluids, e.g. BAL, Pleural fluid, Saliva etc.

2.2 Sample amount: > 5 ml

2.3 Storage conditions and period: Refrigerated (7 days), Frozen (> 7 days)

2.4 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, 10N NaOH, 1X PBS (pH 6.8~7.2)

2.5 Method

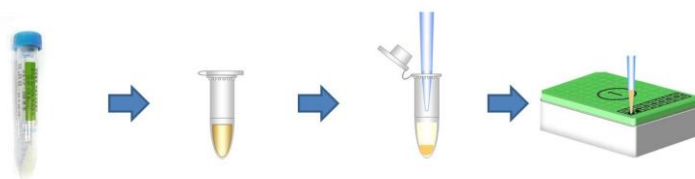
- ① Vortex the sample container hard and transfer 5~10 ml of sample to a conical tube.

Note) If the sample is viscous, transfer 200 µl 10 N NaOH and incubate at room temperature for 15 minutes. If the incubated fluid includes floating particles, remove the particles before use.

- ② Centrifuge at 3,000 rpm for 20 minutes and completely remove the supernatant.
- ③ Add 1 ml of 1x PBS and vortex for 30 seconds and transfer to a sterilized 1.5 ml microcentrifuge tube. Centrifuge the sample at 9,000 rpm for 5 minutes and completely remove the supernatant.

Note) If a white layer forms above the cell pellet, remove the layer with the supernatant and leave the cell pellet.

- ④ Transfer 400 µl of Resuspension Buffer, 1x PBS or normal saline and completely dissociate the cell pellet using a pipette.
- ⑤ Briefly spin-down at 6,000 rpm for 5 seconds. The sample is now ready to be used.



(Sample loading well or tube)



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3 Processing Tissue

3.1 Sample amount: 50 mg ~ 100 mg

3.2 Storage conditions and period: Refrigerated (7 days), Frozen (> 7 days)

3.3 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, shaking incubator (60°C)

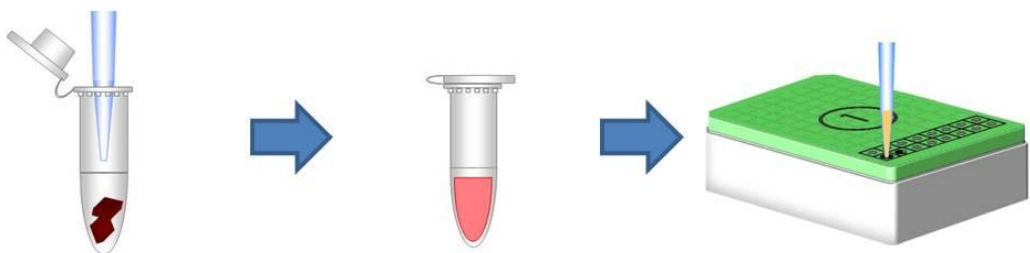
3.4 Method

- ① Transfer 50 mg-100 mg tissue sample into a sterilized 1.5 ml microcentrifuge tube.
- ② Place 20 μl of Proteinase K (20 mg/ml, sold separately), and 400 μl of Tissue lysis buffer (sold separately) into the sample tube and incubate the sample until the tissue is completely lysed.

Note) Complete lysis usually occurs in 1 hour, but the sample may be incubated overnight if necessary.

- ③ Centrifuge at 8,000 rpm for 2 minutes and use the supernatant for the experiment.

Note) If a white layer forms above the pellet, DO NOT disturb the pellet while taking the supernatant.



(Sample loading well or tube)



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4 Processing FFPE (Formalin-Fixed Paraffin-Embedded Tissue)

4.1 Sample Amount: > 20 mg

4.2 Storage conditions and period: Room temperature

4.3 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, shaking incubator (56°C, 60°C)

4.4 Method

- ① Transfer 20 mg FFPE sample into a sterilized 1.5 mL microcentrifuge tube.
- ② Add 1200 μL xylene into the sample tube and briefly vortex the sample. Then place the tube in a 56°C shaking incubator (approx. 1 hour).
- ③ If paraffin is melted thoroughly, briefly vortex the sample tube then centrifuge the tube at 13,000 rpm for 5 minutes at room temperature.
- ④ Completely remove the supernatant and add 1 mL of 100% ethanol in the sample tube. Briefly vortex the tube and then centrifuge at 13,000 rpm for 5 minutes at room temperature.
- ⑤ Completely remove the supernatant and add 1 mL of 80% ethanol in the sample tube. Briefly vortex the tube and then centrifuge at 13,000 rpm for 5 minutes at room temperature.
- ⑥ Completely remove the supernatant and add 1 mL of 60% ethanol in the sample tube. Briefly vortex the tube and then centrifuge at 13,000 rpm for 5 minutes at room temperature.
- ⑦ Completely remove the supernatant and leave the tube open at room temperature for 10 minutes.
- ⑧ Add 1 mL of 1x PBS buffer into the sample tube and briefly vortex the tube.



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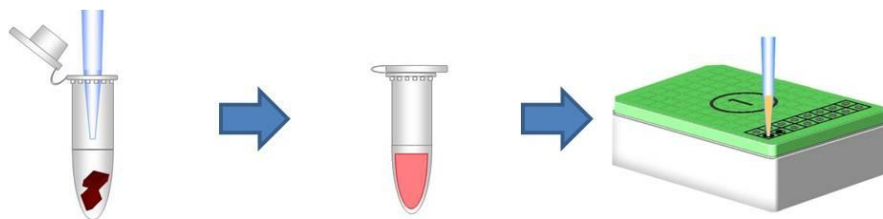
Centrifuge at 13,000 rpm for 5 minutes at room temperature and completely remove the supernatant.

- ⑨ Place 20 μl of Proteinase K (20 mg/ml, sold separately), and 400 μl of Tissue lysis buffer (sold separately) into the sample tube and incubate the sample tube in a 60°C shaking incubator until the tissue is completely lysed.

Note) Complete lysis usually occurs in 3 hour, but the sample may be incubated overnight if necessary.

- ⑩ Centrifuge at 8,000 rpm for 2 minutes and use the supernatant for the experiment.

Note) If a white layer forms above the pellet, DO NOT disturb the pellet while taking the supernatant.



(Sample loading well or tube)



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5 Processing Urine

5.1 Sample Amount: > 10 mL (first morning urine is recommended)

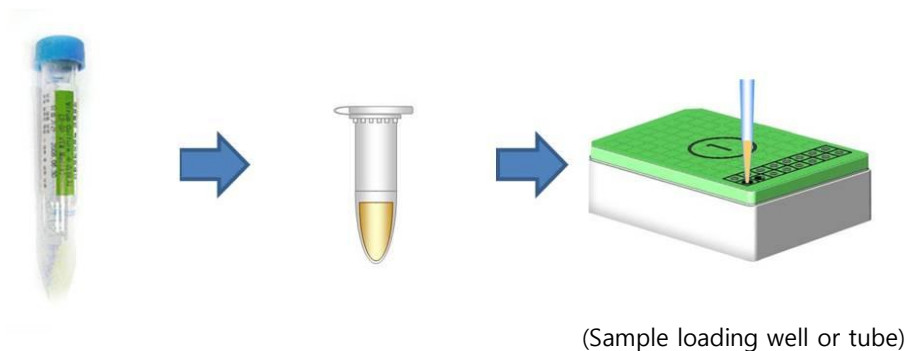
5.2 Storage conditions and period: Refrigerated (7 days), Frozen (> 7 days)

5.3 Materials: Swing rotor centrifuge (up to 3,000 rpm), vortex mixer

5.4 Method

- ① Centrifuge the sample tube at 3,000 rpm for 20 minutes and remove the supernatant.
- ② Transfer 400 μL of Resuspension Buffer, 1x PBS or normal saline and completely dissociate the cell pellet using a pipette.

Note) If excess sediment causes viscosity issues, buffer volume can be increased up to three times more than the sediment volume.





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6 Processing Bacterial Swabs

6.1 Sample unit: 1 swab

6.2 Sample collection method

Take a sterilized brush for the use of specific sample collection, and insert (up to 1 inch) into the urethra (use urological use-only brush), Vaginal/Cervical, throat (use respiratory use-only brush) etc. Smoothly twist to coat the brush, and keep the brush in its container.

6.3 Storage conditions and period: Refrigerated (7 days), Frozen (> 7 days)

6.4 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, 1X PBS (pH 6.8~7.2)

6.5 Method

- ① Vortex the sample container hard for 3 minutes. Transfer 500 μl of fluid into a sterilized 1.5 ml microcentrifuge tube.

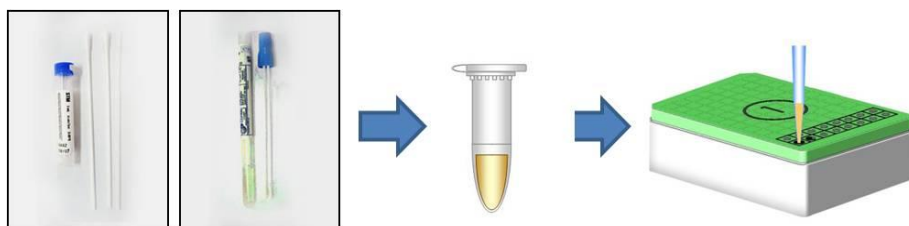
Note) If the sample is dried in the absence of buffer, the sample cannot be used for a diagnostic purpose.

Note) If viscous samples cause tips to clog, add 2 ml PBS buffer and vortex the sample before use.

- ② Centrifuge the sample at 13,000 rpm for 5 minutes and completely remove the supernatant.

- ③ Transfer 400 μl (200 μl for *ExiPrep*TM16 Plus instrument) of Resuspension Buffer, 1x PBS or normal saline and completely dissociate the cell pellet using a pipette.

Caution) Hard particles that cannot be resuspended may cause tips to clog during the nucleic acid extraction process. Only use completely resuspended solutions.





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7 Processing Viral Swabs

7.1 Sample unit: 1 swab

7.2 Sample collection method

Take a sterilized brush for the use of specific sample collection, and insert (up to 1 inch) into the orifice of choice. Smoothly twist to coat the brush, and keep the brush in its container.

(Nasal swabs) Insert deeply (1/3 position between a nose and a ear) into the nostril while twisting to collect enough sample to be noticeably visible

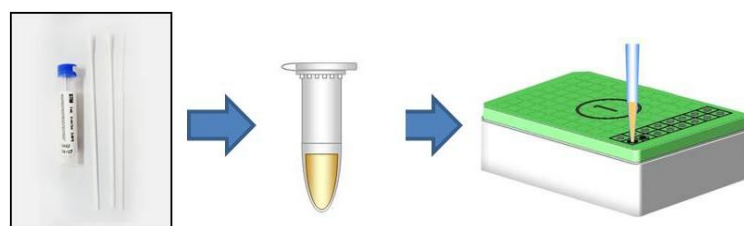
(Throat swabs) Insert deeply into the throat or surface of tonsils while twisting to collect enough sample to be noticeably visible (Take caution not to stimulate the uvula).

7.3 Storage conditions and period: Refrigerated (3 days), Frozen (> 3 days)

7.4 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer

7.5 Method

- ① Vortex the container hard so that viral particles on the swab are transferred into the viral transport media, 1x PBS or normal saline.
- ② Transfer 500 μl of liquid into a sterilized 1.5 ml microcentrifuge tube and centrifuge at 6,000 rpm for 5 seconds.
- ③ The supernatant may be used for ensuing experiments.



(Sample loading well or tube)



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8 Processing Stool

8.1 Sample amount: 50 mg ~ 200 mg / 200 μl

8.2 Sample collection method

Take a 1~2 g / 2 ml portion of stool where pus, blood or mucus is present and transfer into a dry, clean container.

8.3 Storage conditions and period: Refrigerated (3 days)

8.4 Materials: Table top centrifuge (up to 13,000 rpm), vortex mixer, shaking incubator (60°C), Stool lysis buffer (sold separately), Proteinase K (sold separately)

8.5 Processing Stool for Virus

- ① Transfer 50~200 mg sample into a sterilized 1.5 ml microcentrifuge tube and add 400 μl Stool lysis buffer.
- ② Vortex the tube hard for 3 minutes.
- ③ Centrifuge at 6,000 rpm for 5 seconds and use the supernatant for ensuing experiments.

8.6 Processing Stool for Bacteria

- ① Transfer 50~200 mg sample into a sterilized 1.5 ml microcentrifuge tube and add 400 μl Stool lysis buffer with 10 μl Proteinase K (40 mg/ml).
- ② Vortex the tube hard for 1 minute. Then place the tube in a 60°C shaking incubator until the sample is completely dissociated (approx. 30 minutes)
- ③ After complete lysis, centrifuge at 12,000 rpm for 5 minutes.
- ④ The supernatant may be used for ensuing experiments.



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Examination	Sample Type	Examination	Sample Type
Tuberculosis	Sputum	Influenza/ Adenovirus	Nasal Swab
	BAL		Throat swab
	Fluids		
	Tissue		
STI	Urine	Pneumonia	Sputum
	Swab		Fluids
Gastrointestinal Diseases	Stool	Norovirus	Stool
	Swab		Swab
HSV	Swab	HPV	Swab
	CSF		

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