Product code:69503

Research Use Only

COSMO BIO CO., LTD.
Inspiration for Life Science

DNA / RNA Extraction Kit

MORA- EXTRACT

Instruction Manual

Table of Contents

1: Kit contents (for 50 times)

1)	Lysis buffer (non-medical deleterious substance *) (containing 0.72% of 2-mercaptoethanol)	10 mL × 1 bottle
2)	Tube containing beads	50 tubes
3)	SDS solution^{+}	10 mL × 1 bottle
4)	Phenol mixture (non-medical deleterious substance *) (containing 42% of phenol)	20 mL × 1 bottle
5)	DEPC-treated water (for dissolving purified nucleic acid)	10 mL × 1 bottle
6)	2-mL tube for laboratory	50 tubes
7)	Paper stand for laboratory	1 unit
8)	Instruction	1 set

* Description based on Japanese legal regulations.

2: Purpose of use

Nucleic acid (DNA/RNA) extraction from microorganisms
(This kit was developed in order to easily extract and purify nucleic acids of pathogen, etc. included in various samples from them as a prior step of gene amplification. Especially, it has excellence performance for trace amount of specimens.)

3: Reagents and equipment necessary for operation

[Reagents/supplies]
1) 99% Ethanol
2) 70% Ethanol (mixture of 99% ethanol and DEPC-treated water or sterile distilled water in the ratio of 7 to 3)
3) Sterile 2-mL microtube
4) Sterile tip (for 1,000 μL and 200 μL; Filter tips are recommended)
5) RNase-free 10 mM Tris – 1mM EDTA pH 8.0 or pH 7.6 (TE buffer) (for storage of extracted DNA/RNA)

[Equipment]
1) Water bath or heat block
(Heat to 70°C [or 90°C as necessary] before starting operation. Use a heat block for 90°C.)
2) Homogenizer (The following device is recommended.)
- Disruptor Genie (Scientific Industries; M&S Instruments Inc.)
3) Micropipette (for 200 – 1,000 μL, for 20 – 200 μL, and for 2 – 20 μL)
4) Mixer (Vortex)
5) High speed micro-centrifuge
6) Ultraviolet-visible Spectrophotometer (for quantification of extracted DNA/RNA)
7) Tabletop-type mini centrifuge (to drop liquid on sidewalls or back of a lid)

4: Basic operations

In case of a sample from clinical specimens, foods, or environment, it should be pretreated by such as suspension, homogenization, precipitation, or concentration separately for each sample. The basic operations after such pretreatment are as follows.

1) Take the bacteria solution, which is prepared by suspending bacterial culture or cells in such as saline, or the pretreated sample into a sterilized 2-mL microtube, and centrifuge it at 15,000 rpm (or not less than 12,000 ×G) for 3 to 5 minutes. Extend to 15 to 20 minutes when a precipitation enhancer is necessary.

2) After centrifugation, discard the supernatant to obtain precipitate or liquid, add 150 µL of lysis buffer to it, and mix well to suspend.
(If any precipitate generates in the lysis buffer, dissolve the precipitate by allowing it to warm to room temperature before use.)

3) Transfer the suspension into the tube containing beads, heat at 70 to 90°C*[1] for 10 minutes to facilitate bacteriolysis.

*1: In case of extraction of RNA, treat at 70°C. Although DNA usually can be extracted at 70°C, the disruption efficiency for gram-positive bacteria will improve in treatment at 90°C.

4) After heating treatment, disrupt for 2 minutes with Disruptor Genie (Scientific Industries).
In case a homogenizer is not available, DNA/RNA extraction also can be achieved by treatment with Vortex for 2 minutes*[2].

*2: The disruption conditions other than those mentioned above may damage the tube containing beads. Especially, vigorous shaking should be avoided in the device dedicated for cell-disruption with beads.

5) After disruption, drop the liquid on the lid by flushing of the centrifuge or with a tabletop-type mini centrifuge, add 200 µL of SDS solution^{+}, mix it, and heat again at 70°C for 10 minutes.
(If any precipitate generates in the SDS solution^{+}, dissolve the precipitate by allowing it to warm to room temperature before use.)

6) Add 400 µL of phenol mixture*[3], mix it, centrifuge at 15,000 rpm for 3 minutes, collect the upper layer*[4] into the attached 2-mL tube for laboratory (crude nucleic acid purified)

*3: Take only the yellow liquid in the lower layer not to take the transparent liquid in the upper layer.

*4: Take only the transparent liquid in the upper layer to prevent contamination with the yellow phenol layer and the denatured middle layer (white).

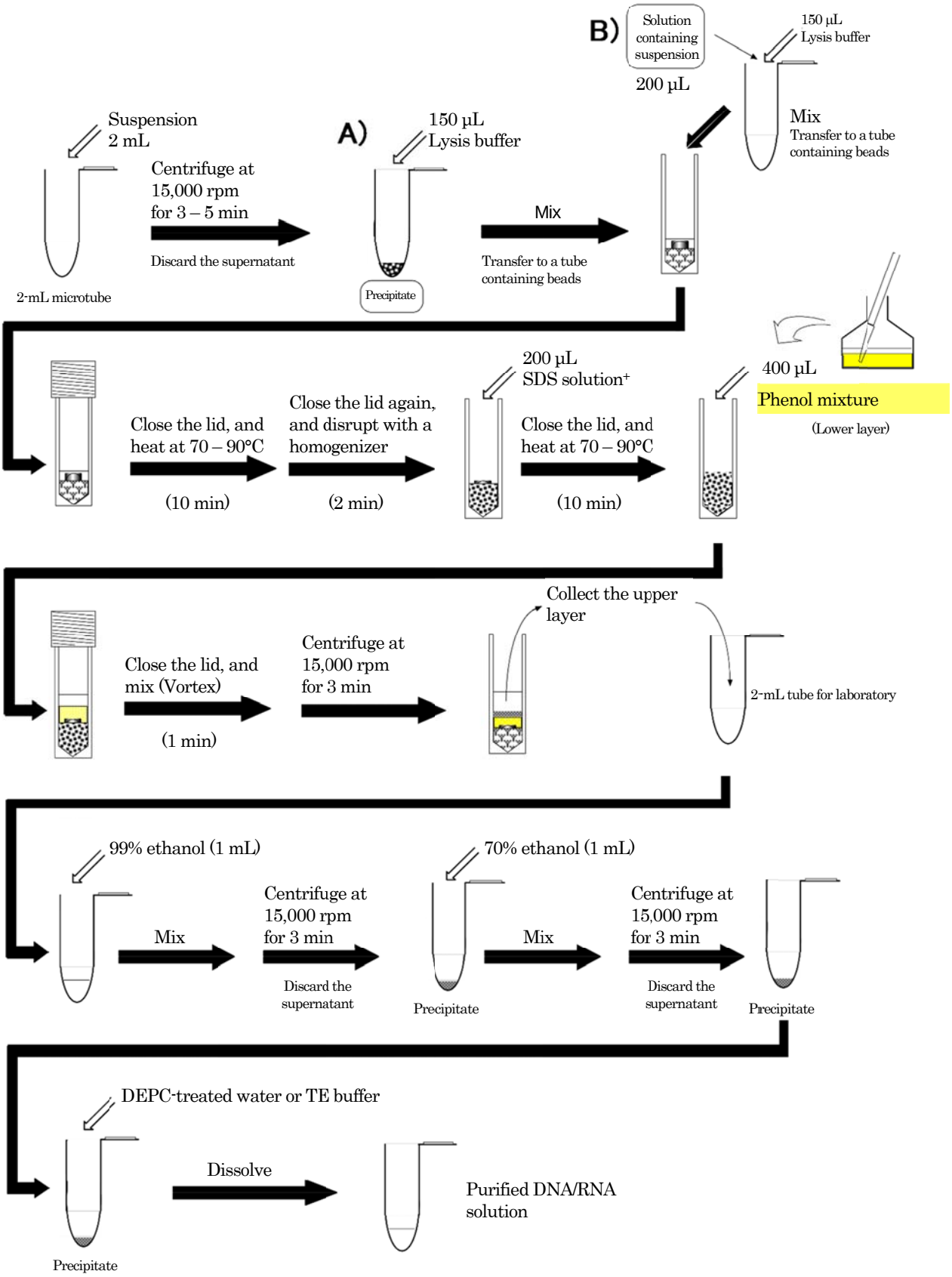
7) Add 1 mL of 99% ethanol to the crude nucleic acid purified (use of a commercially available coprecipitation agent is recommended in case of less microbial count or for beginners), mix it, and centrifuge at 15,000 rpm for 3 minutes. Discard the supernatant*[5], add about 1 mL of 70% ethanol, mix it, and centrifuge at 15,000 rpm for 3 minutes again. Then discard the supernatant, and dry the precipitate.
Purification with magnetic beads followed by washing is also useful instead of centrifugation.

*5: Remove the supernatant completely so that the components of the lysis buffer do not remain.

8) Add 200 µL of DEPC-treated water (for immediate use) or TE buffer (for storage) to the dried precipitate, and dissolve it (purified nucleic acid).

The basic operations flow is shown in the following page.
Also refer to the protocol for each specimen as the appropriate liquid volume in the pretreatment or the basic operations may change depend on the specimen type.

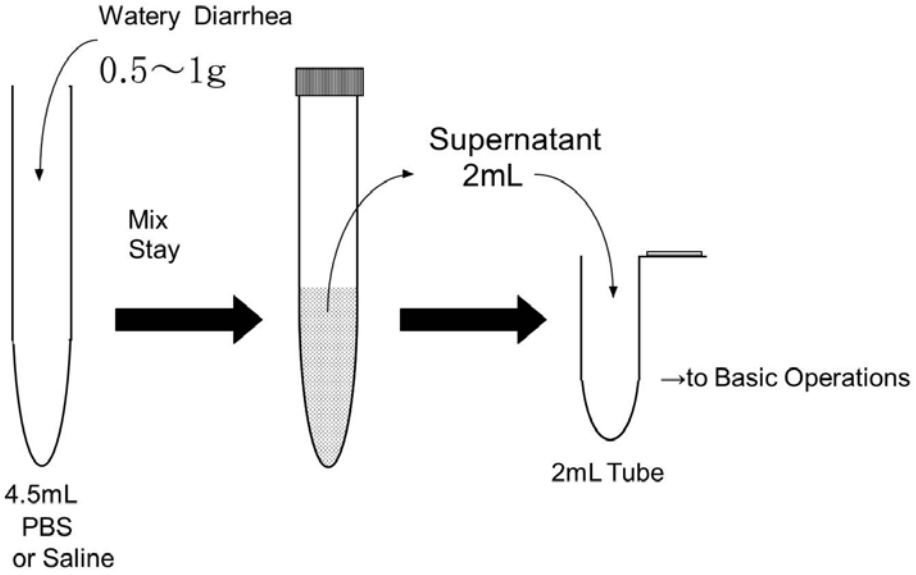
5: Basic operations flow



6. Protocols for Nucleic Acids Extraction by samples

A) DNA / RNA extraction of microorganism from feces samples

A-1) DNA / RNA extraction of bacteria from watery diarrhea



Pre-treatment of watery diarrhea

Collect 0.5-1g of watery diarrhea and mix well with 4.5-9 mL of PBS (or saline).
*This operation enables to move sample suspension safely to 2mL tube and improve recovery by homogenizing diarrhea solution.

Preparation of watery diarrhea samples

1. Transfer 2mL of watery diarrhea suspension to experimental tube (2mL capacity), treat with high speed microcentrifuge at 15000rpm / 3min and discard supernatant. Add 150μL of lysis buffer to remaining precipitate and mix well. Whole suspension is transferred to tube containing beads.
*As lysis buffer may precipitate in low temperature, it is recommended to reconstitute at room temperature before use and dissolve precipitate if any.

2. Heat the suspension at 70℃ / 10min and enhance bacteriolysis.
*In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

3. Close the lid of tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
Even disruption device is not available, it is possible to extract DNA / RNA by treatment with Vortex at 2 min.

4. After disruption process, add 200μL of SDS solution and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

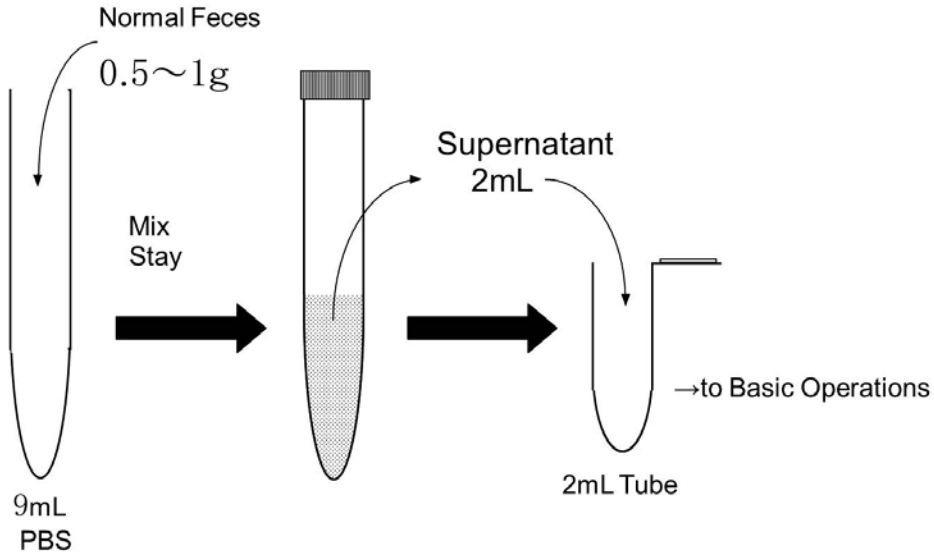
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

A-2) DNA/RNA extraction of bacteria from normal feces



Pre-treatment of normal feces

Collect 1g of feces sample and suspend well with 9mL of PBS or saline. Leave to stand for 1-3 min and collect 2mL of supernatant as sample for bacteria test (this operation enables to homogenize feces samples and improve recovery of test except large scale of food test.

Sample preparation of normal feces

1. Transfer 2mL of feces suspension to experimental tube (2mL capacity), treat with high speed microcentrifuge at 15000rpm / 3min and discard the supernatant.

2. Add 150μL of lysis buffer to remaining precipitate and mix well. Whole suspension is transferred to the tube containing beads.

3. Enhance bacteriolysis by heating at 70-90℃/ 10min
* In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

4. Close lid of tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

5. After the process of disruption, add 200μL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

6. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

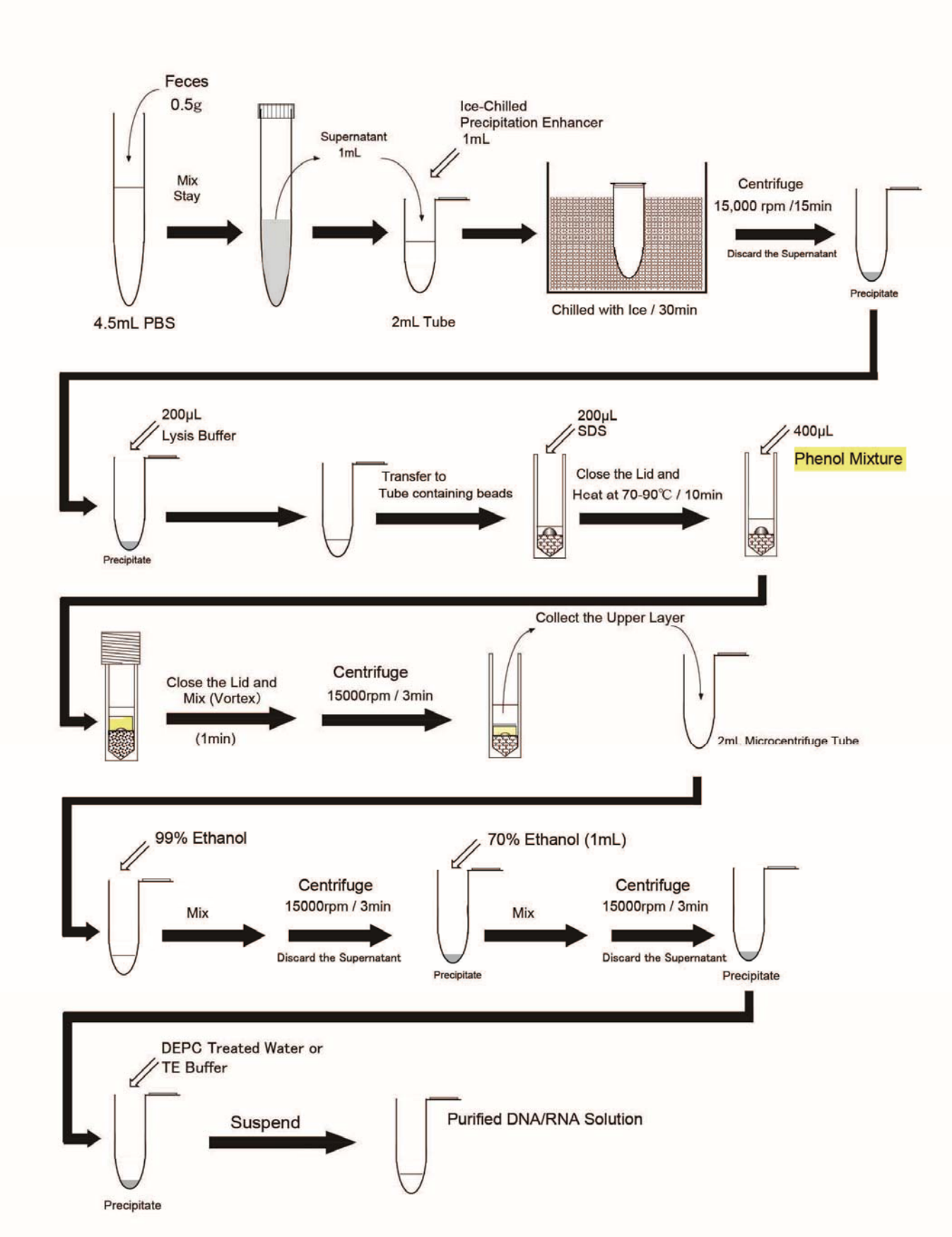
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

A-3) Viral RNA Extraction from diarrheal stool



Pre-treatment of diarrheal stool samples

1. Collect 0.5-1g of diarrheal stool and suspend well with 4.5-9mL of PBS or (or saline). Leave to stand for a while and collect 1mL of supernatant as sample for viral RNA test (this operation enables to homogenize feces samples and improve recovery). It is possible to apply 2mL of remaining aliquot to bacteria test.

2. For the test of RNA virus, transfer 1mL of the supernatant to experimental tube (2mL capacity), add same volume of precipitation enhancer and chilled with ice staying 90℃ or leave at 4℃ / overnight.

3. Centrifuge at 15000rpm / 20min / 4℃ and discard the supernatant.

Preparation of diarrheal stool samples

4. Add 200μL of lysis buffer and suspend. Transfer whole suspension to tube containing beads and add 200μL of SDS solution^{+}. Mix well and stay at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect the supernatant as crude RNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

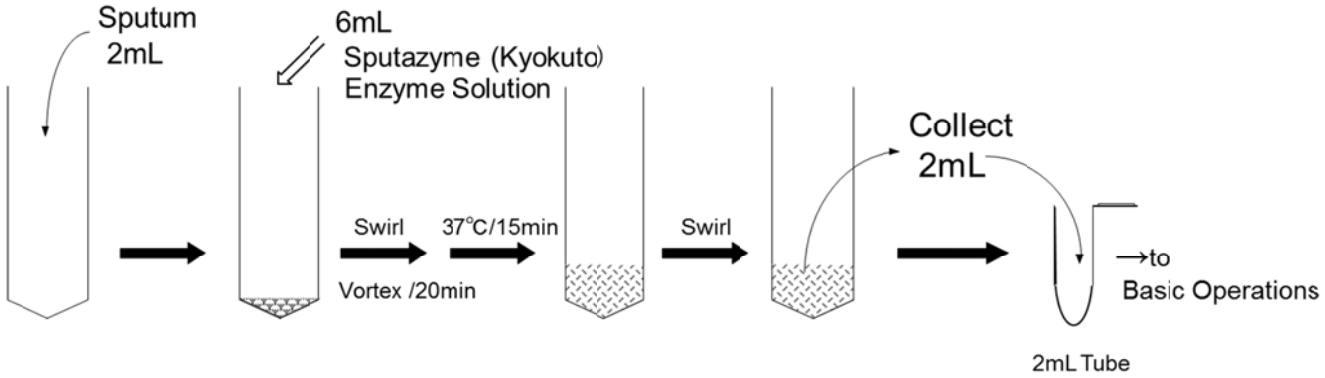
2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

B) Pathogenic DNA / RNA derived from sputum or pharyngeal scrap samples

B-1) Extraction of bacteria or mold DNA/RNA from sputum



Pre-treatment of sputum

1. Collect about 2mL of sputum and mix with 3×volume of "Sputazyme (Kyokuto) and decompose viscose substance with the enzyme rotating at Room Temperature / 15min.

2. Transfer reaction mixture to experimental tube (2mL capacity), centrifuge at 15000rpm / 3min and discard supernatant. (aliquot in step 1 is possible to apply to virus test).

Preparation of Sputum samples

3. Add 150µL of lysis buffer to pre-treated sputum precipitation and suspend well.. Transfer whole volume to tube containing beads and stay 70℃ / 10min to enhance bacteriolysis.

*Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitate

* *In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

4. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

5. After the process of disruption, add 200µL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

6. Add 400µL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

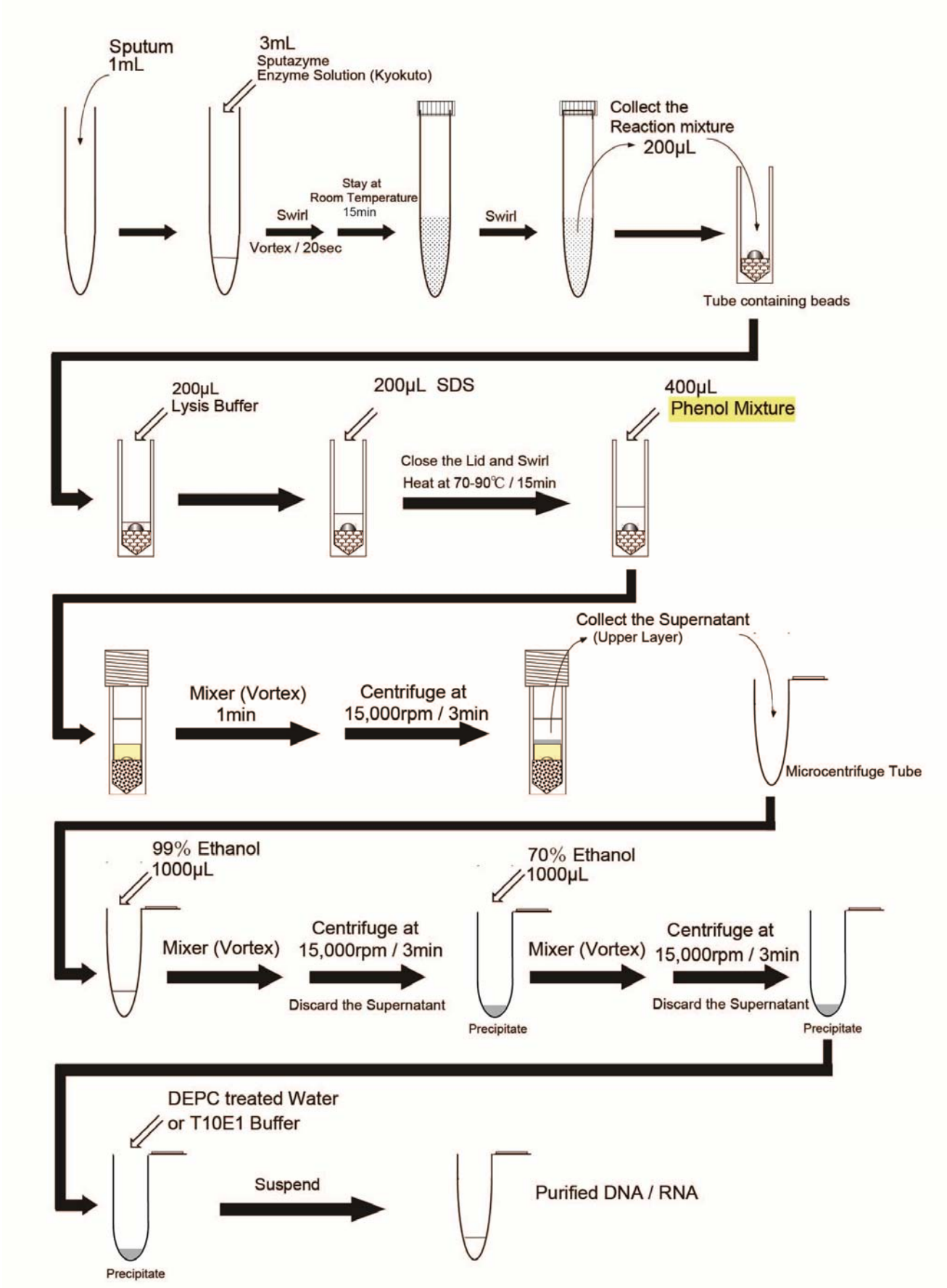
Purification with Ethanol Precipitation Method

1. Transfer 200µL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000µL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000µL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200µL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

B-2) Extraction of viral RNA from sputum



Pre-treatment of Sputum

1. Collect 1mL of with sampling tube (14mL capacity) and add 3×volume of Sputazyme (Kyokuto). Mix well, incubate at 15min / Room temperature and use 200μL for virus test.

Preparation of Sputum samples

2. Pre-treated sputum sample is transferred to beads packed tube. Add 200μL of lysis buffer and 200μL of SDS solution^{+} and heat at 70℃ / 15min.

3. Add 400μL of phenol mixture, shake 1min with mixer or Vortex.

4. Centrifuge at 15000rpm/5min and collect 200μL of the supernatant as crude DNA/RNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

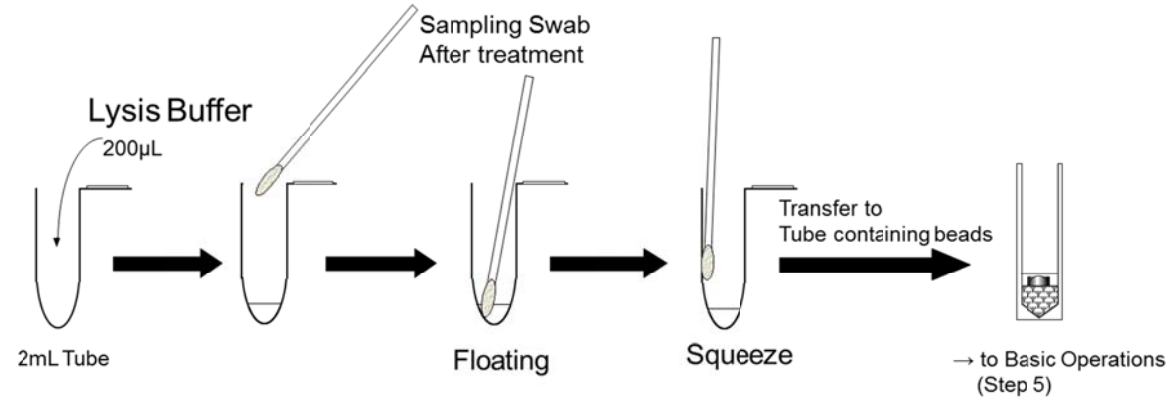
Purification with Ethanol Precipitation Method

5. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

6. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
7. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

B-3) Extraction of bacterial or viral DNA/RNA from pharyngeal scrap samples



1. Transfer sample material to experimental tube (2mL capacity) containing 200μL of lysis buffer and squeeze well with cotton swab.

2. Remove swab, transfer squeezed liquid to beads packed tube and enhance bacteriolysis staying at 70℃/10min.
*Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitant.
** In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

3. Close the lid of tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min..

4. After the process of disruption, add 200μL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.
*In case volume of supernatant is less than 100μL, add more of 200μL lysis buffer and repeat operation of step 5.

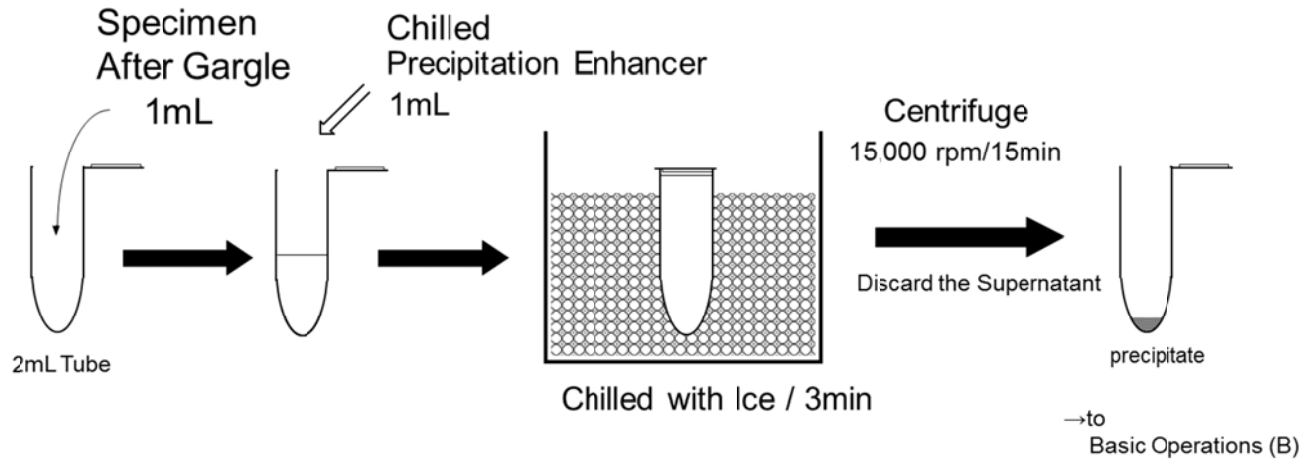
Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2ml capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

B-4) Extraction of bacterial or viral DNA/RNA from specimen after gargle.



1. Transfer 1ml of sample, add chilled precipitation enhancer and stay at 4℃ / 90min.

2. Centrifuge at 15000rpm/15min and discard supernatant. Add 15μL of lysis buffer to remaining precipitate, mix well and transfer whole volume to beads packed tube. Enhance bacteriolysis staying 70℃ / 10min.

3. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries).
Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. After the process of disruption, add 200μL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex).
Collect supernatant as crude DNA solution.

Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

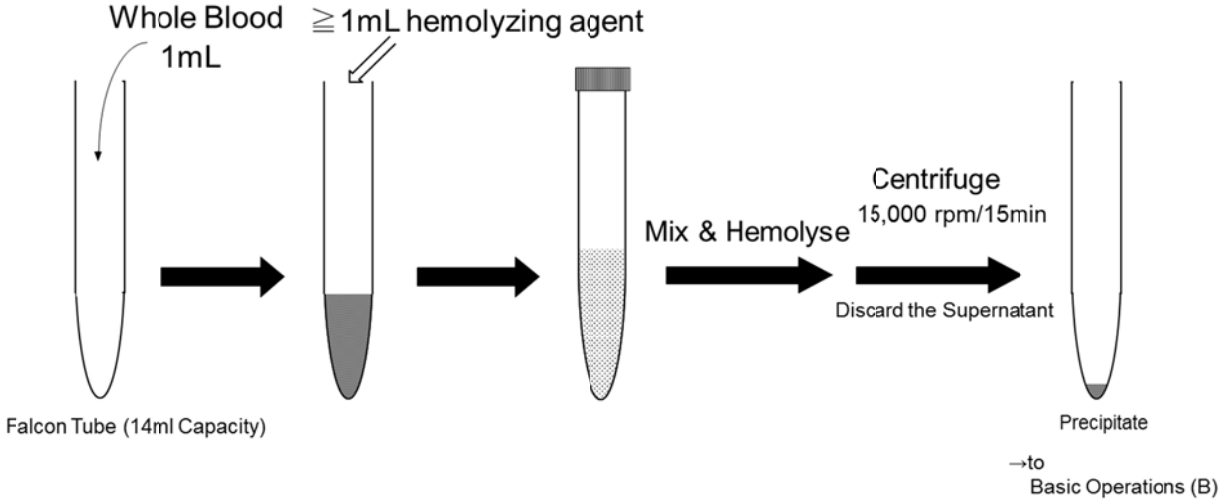
Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

C) Extract of pathogenic DNA / RNA from blood samples

C-1) Extract of pathogenic DNA/RNA from whole blood samples



Pre-treatment of blood (VNC or samples from severe sepsis requiring urgency containing large amount of bacteria)
Transfer 1mL of whole blood to falcon tube (14mL capacity) and add 1mL of hemolyzing agent. Mix well to dissolve red blood cells and centrifuge at 4500x g (5000rpm)/15min.

Blood
1. Discard the supernatant, add 150µL of lysis buffer to remaining precipitate and suspend well. After the operation, transfer whole suspension to tube containing beads.

2. Enhance bacteriolysis by heating at 70-90℃ / 10min.
* In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

3. Close the lid of tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. After the process of disruption, add 200µL of SDS solution+ and enhance bacteriolysis at 70℃ / 10min.

5. Add 400µL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

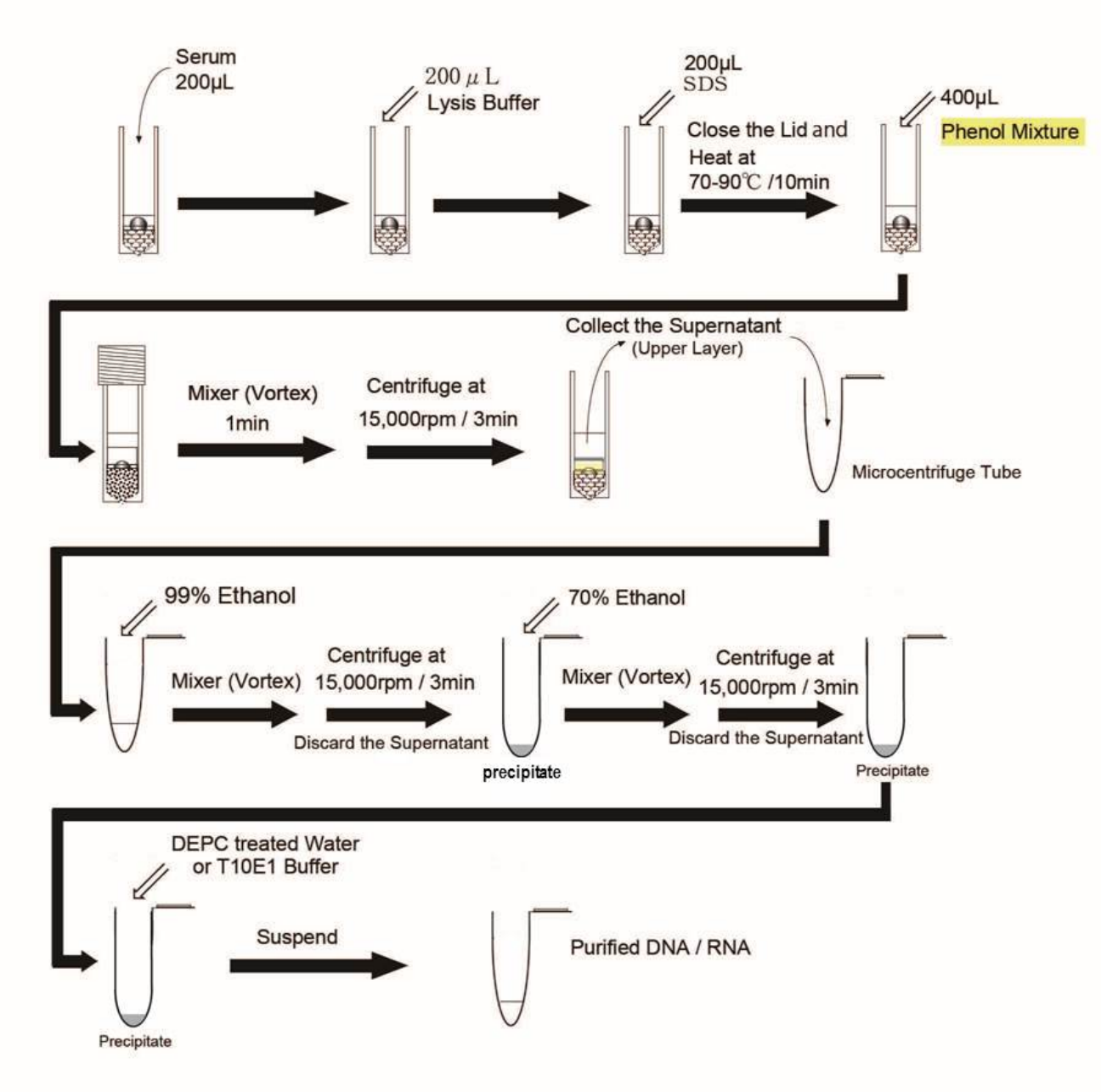
Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method
1. Transfer 200µL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000µL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000µL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200µL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

C-2) Extraction of viral DNA/RNA from serum samples



1. Transfer 200μL of serum to beads packed tube, mix well adding 200μL of lysis buffer and 200μL of SDS solution+

2. Heat at 70℃ / 10min, add 400μL of phenol solution and shake 1min with mixer or vortex.

3. Treat with high speed microcentrifuge at 15000rpm / 3min and collect the supernatant as crude DNA/RNA solution.

Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

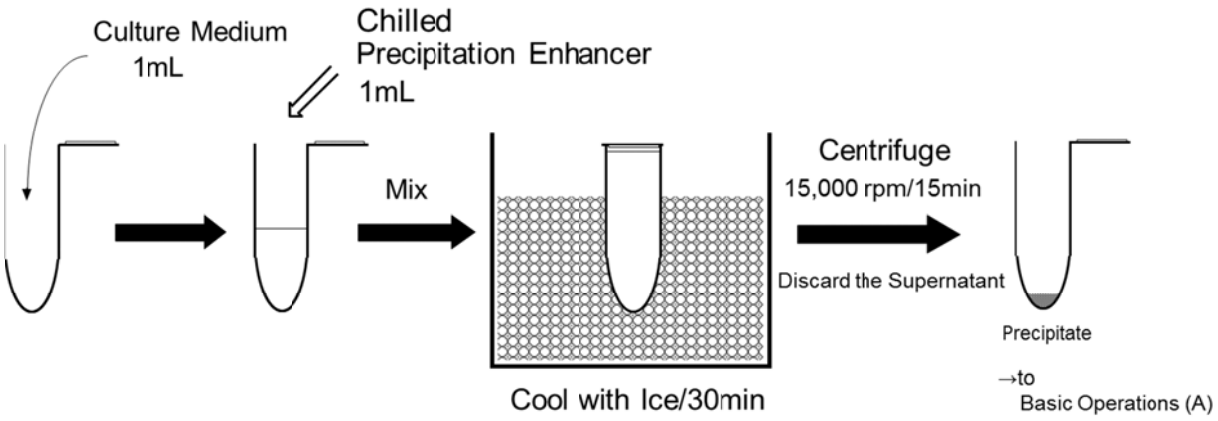
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

D) Extraction of pathogenic DNA/RNA from colony or culture medium

D-1) Extraction of bacteria or mold DNA/RNA in culture bottle with small numbers of fungi..

Operate nucleic acid extraction according to the protocol below when the sign of culture positive is observed in bloodculturing.



1. Transfer 1ml of culture medium to experimental tube (2mL capacity), add precipitation enhancer and chilled at 30nin or stay at 4 ℃/ 90min.

2. Centrifuge at 12000g (15000rpm) / 4℃ /12min and discard the supernatant.

3. Add 150μL of lysis buffer to remaining precipitate and mix well with pipette. Enhance bacteriolysis by staying at 20-90℃ / 10min.
*Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitant.
** In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

4. Transfer whole suspension to tube containing beads and disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even no disruption devices, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

5. After the process of disruption, add 200μL of SDS solution+ and enhance bacteriolysis at 70℃ / 10min.

6. Add 400μL of phenol mixture and shake on the mixer at 1min.
** As there may be a case sediment of Tris buffer is floating in phase of supernatant, handle with care not to suck it with pipette.

7. Centrifuge at 15000rpm / 3min and collect 200 μl of the crude DNA Supernatant.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

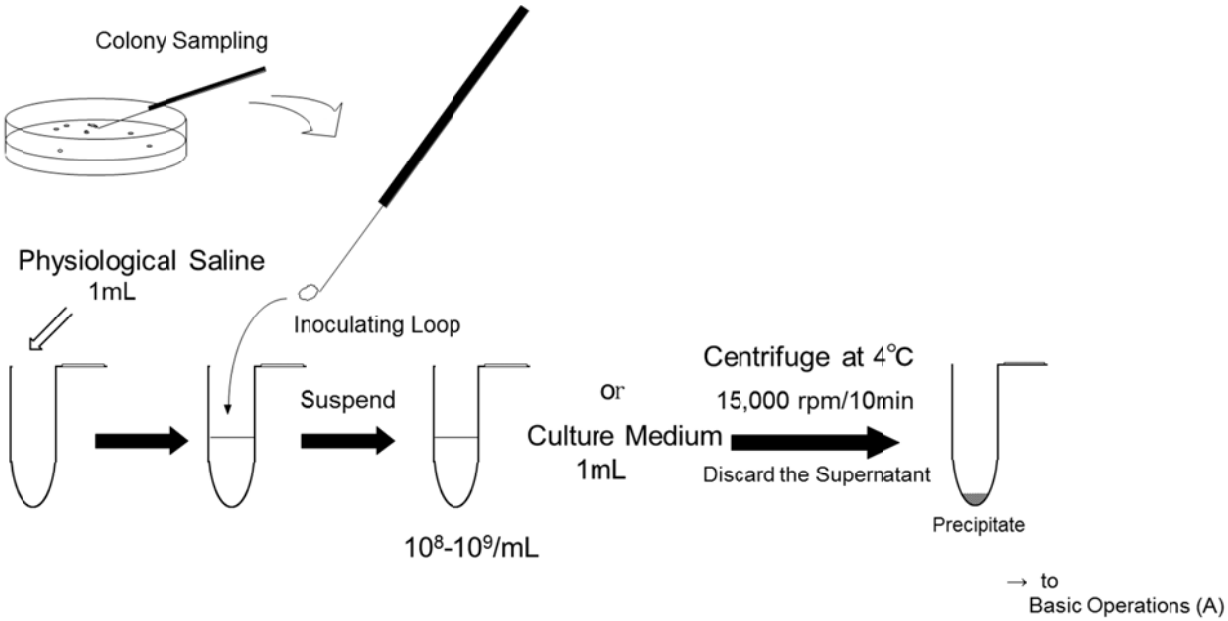
Purification with Ethanol Precipitation Method

1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

D-2) Extract of pathogenic DNA/RNA from colony or culture medium



1. In case of sampling from colony, collect colony using inoculating loop and transfer to mincrocentrifuge tube (sterilized) containing 1mL of saline. As for culture medium, transfer 1ml to Eppendorf tube, centrifuge at 12000g (15000rpm) / 4℃ / 10min and discard the supernatant.

2. Add 150μL of lysis buffer to remaining precipitate, mix well with pipette and transfer to tube containing beads.

3. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. Drop minuscule amount of liquid or bubbles sticking to cap surface with tabletop-type micro-centrifuge (10000rpm/ 2-3 sec). Ass 200μL of SDS solution$^+$ and enhance bacteriolysis by heating at 70℃/ 10min.

5. Add 400μL of phenol mixture and vortex for 1min.
*As upper layer of phenol mixture contains liquid with antioxidant, handle with care not to collect the solution.

6. Centrifuge at 15000rpm / 3min and collect the supernatant as crude DNA solution.

Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

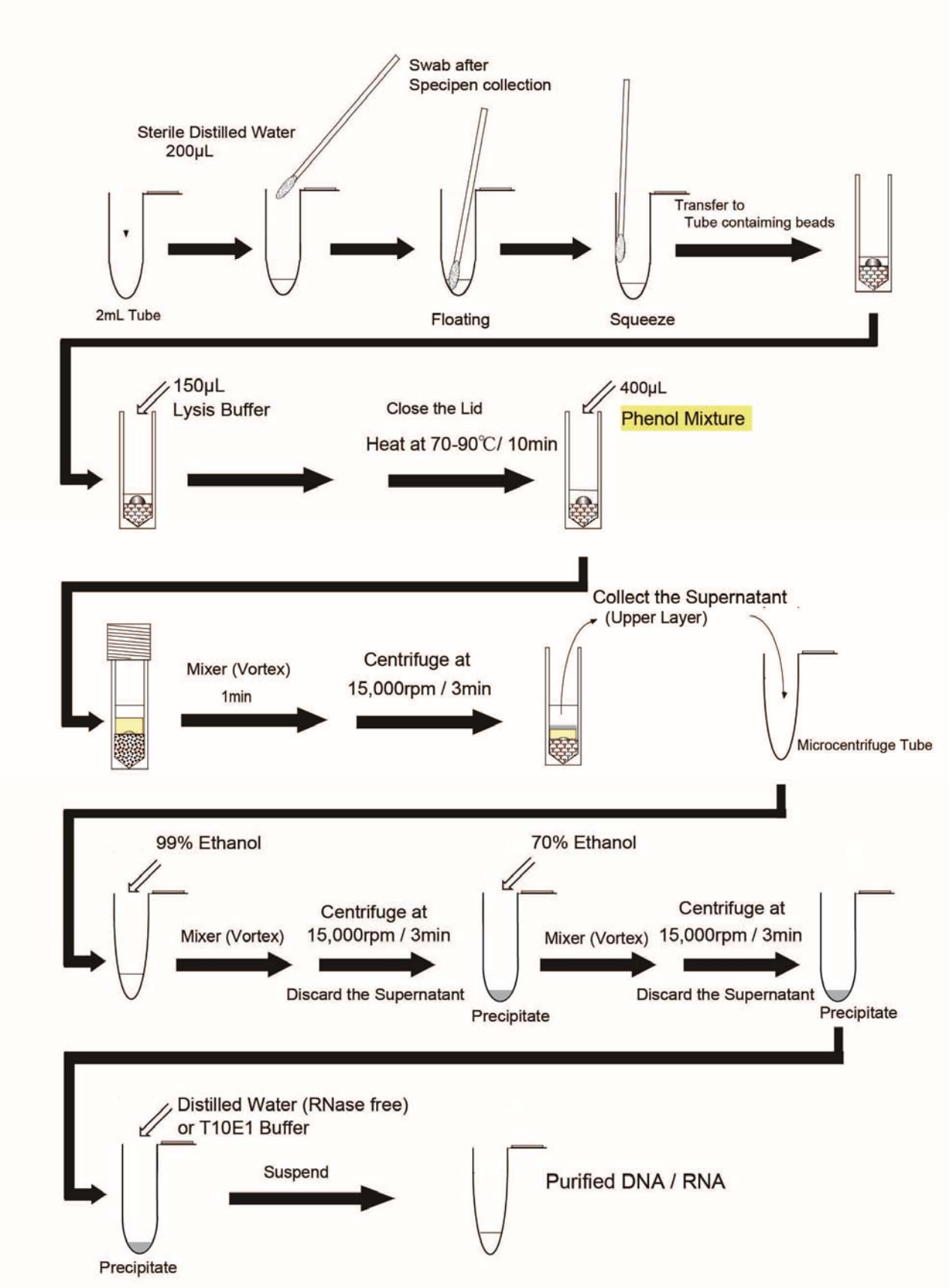
Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

E) Extraction of pathogenic DNA / RNA from urinary samples

E-1) Extraction of bacterial or viral DNA/RNA from secretion of urethra



1. Collect materials with cotton swab and transfer to micro centrifuge tube containing 200μL of sterilized Distilled Water. Wring the cotton well to suspend materials sticking to it.

2. Transfer whole suspension to tube containing beads, add 200μL lysis buffer and mix well.

3. Enhance bacteriolysis by heating at 70-90℃ / 10min.
*In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation. 3 SDS is not required.

4. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2ml capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

E-2) Extraction of bacterial or viral DNA/RNA from first catch urine

1. Collect 2ml of urine and transfer to experimental tube (2mL capacity). Centrifuge at 15000rpm Room temperature / 3min and discard the supernatant.

2. Add 150μL of lysis buffer to remaining precipitate, mix well with pipette and transfer to tube containing beads.
* Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitate.

3. Enhance bacteriolysis staying at 70-90,℃ / 10min.
*In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

4. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

5. After the process of disruption, add 200μL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

6. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

Purification of DNA/RNA
Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method
1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

F) Extraction of pathogenic DNA / RNA from food materials.

Basically, samples are prepared by diluting materials at 10 folds, processed with agitator such as Stomachere and apply to medium. Methods of dilution are varies according to materials (food hygiene law).

F-1) Liquid (not containing solid matter such as fruits juice)

1. Transfer 2mL of suspension to experimental tube (2ml capacity), centrifuge at 15000rpm / 3min and discard the supernatant.

2. Add 150μL of lysis buffer to remaining precipitate and mix well with pipette. Enhance bacteriolysis by heating at 20-90℃ / 10min.

3. Transfer whole suspension to vessel containing beads disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even no disruption devices, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. After the process of disruption, add 200μL of SDS solution+ and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

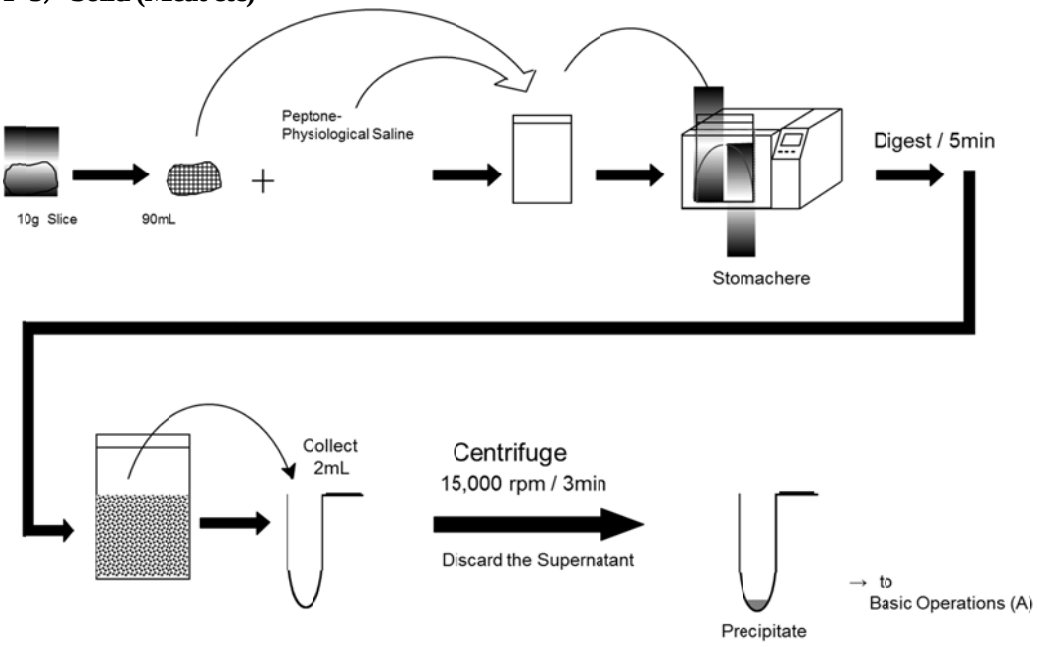
F-2) Semi-Fluid (such as Milk or Ice Cream)

1. Collect 10g of samples and add 90ml of sterilized saline (0.85% NaCl)

2. Process with Stomachere at 5mins.

3. Collect 2ml of samples ant operate with same protocol as liquid samples (from step1)

F-3） Solid (Meat etc)



Chop meat samples finely. Add sterilized saline containing peptone (90mL per 10g sample) and process with Stomachere. Collect 2mL of homogenized material and operate according to same protocol as liquid samples (from step1).

F-4) Marine products ('Raw Oyster etc)

Prepare sterilized phosphate buffer (dissolve 34g of KH_2PO_4 Anhydrous to 500mL of distilled water, adjust pH7.2 adding 1N NaOH 175mL). Mix 10 of samples with phosphate buffer and process with Stomachere. Collect 2mL of homogenized samples and operate according to protocol of liquid samples (from step 1)

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2ml capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

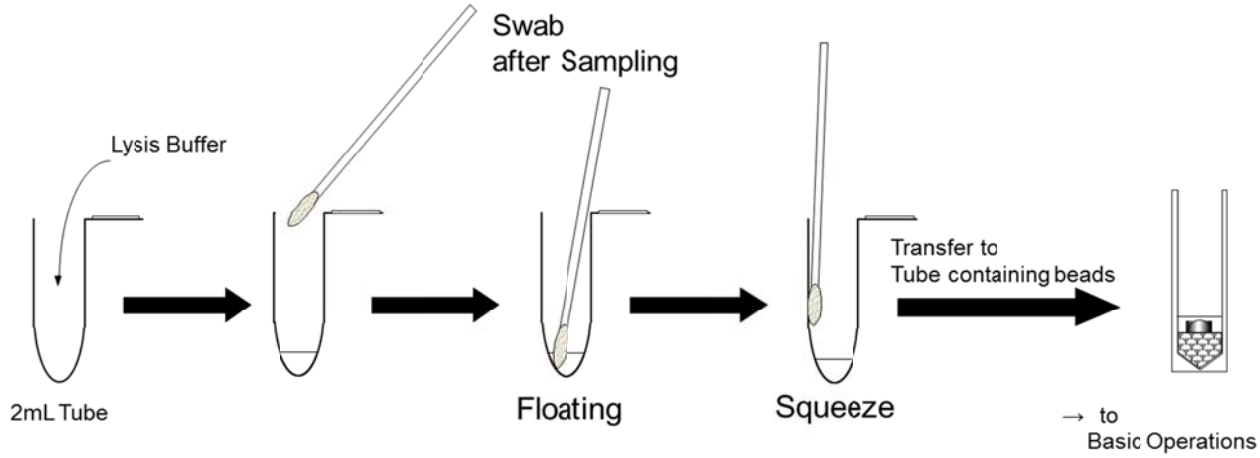
2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

G) Extraction of pathogenic DNA/RNA from for trace amount of samples

(such as ophthalmological specimen)



Pre-treatment

1. Transfer material to sterilized microcentrifuge tube containing 200μL of lysis buffer.
(Wring cotton of swab by stirring. In case treating liquid sample, add maximum 100μL of lysis buffer)

Preparation of Samples

1. Transfer whole suspension to tube containing beads for disruption.

2. Enhance bacteriolysis by heating at 70-90℃ / 10min.
**In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

3. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. After the process of disruption, add 200μL of SDS solution^{+} and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer at 1min
* As there may be cases sediment of Tris buffer is floating in phase of supernatant, handle with care not to suck it with pipette

6. Centrifuge at 15000rpm / 3min and collect 200μL of supernatant as crude DNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

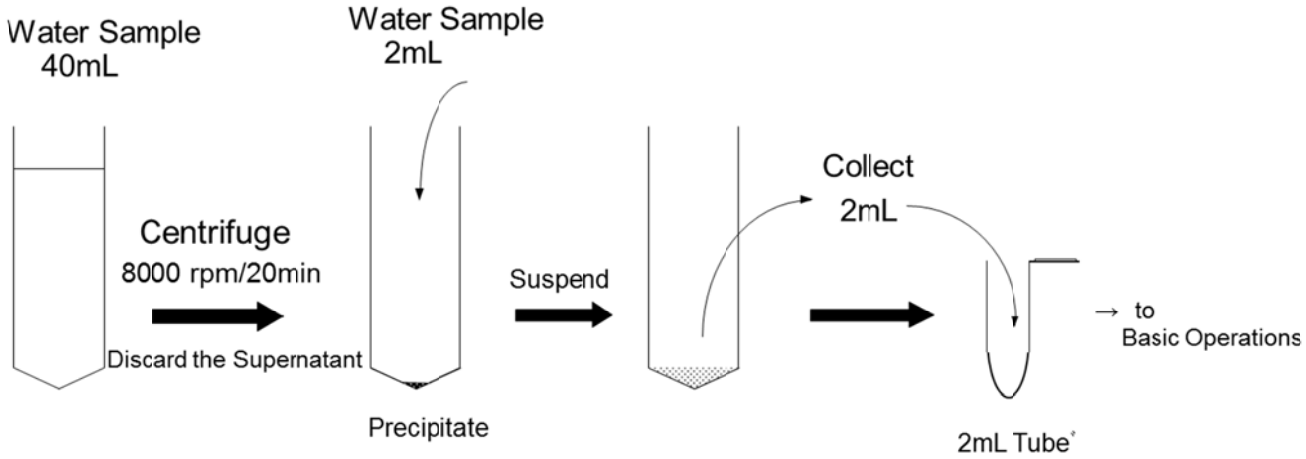
2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.
3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm /230nm and choose appropriate amount for next step of gene amplification process.

H) Extraction of bacterial DNA / RNA from Environmental Water

H-1) Extraction of bacterial DNA / RNA from environmental water heavily polluted (centrifugal concentration)

(Water collected from bath, liver or cool tower)



Concentration procedure

1. Collect 100m of sample, add 40mL of water and centrifuge at 8000rpm/ 20min

2. Discard the supernatant, add 2mL of sample water to remaining precipitate and suspend

3. .Transfer 2mL of suspension to experimental tube (2mL capacity), centrifuge at 15000rpm / 3min and discard the supernatant.

Extraction of DNA

4. Add 150μL of lysis buffer to remaining precipitate and suspend well. Transfer all suspension to tube containing beads.
   * Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitant

5. Enhance bacteriolysis by heating at 70-90℃ / 10min.
   **In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

6. Close the lid of tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries)
   Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

7. After the process of disruption, add 200μL of SDS solution+ and enhance bacteriolysis at 70℃ / 10min.

8 Add 400μL of phenol mixture and shake on the mixer at 1min (or centrifuge at 15000rpm / 3min after treatment with Vortex). Collect supernatant as crude DNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

1. Transfer 200μL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000μL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000μL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).

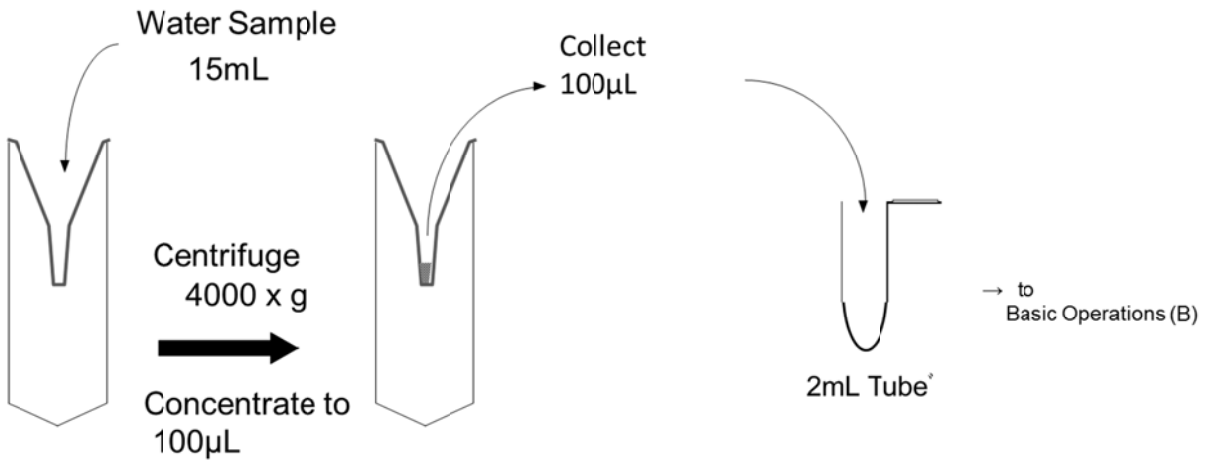
*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200μL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm/230nm and choose appropriate amount for next step of gene amplification process.

H-2) Extraction of DNA / RNA from Clean Water (filtration enrichment)

(Methods for test of trace amount of bacterial contamination of Samples from Tap Water or Distilled Water)



Concentration procedure

1. Transfer 15ml of water sample to concentration tube (ex. Millipore) and operate according to protocol.

2. Adjust final volume to 100µL (applox 150 fold)

3. Transfer to sterile microcentrifuge tube

Extraction of DNA

4. Add 150µL of lysis buffer to remaining precipitate and suspend well. Transfer all suspension to tube containing beads.
   * Lysis buffer may precipitate under environment of low temperature. In such case, reconstitute it at room temperature to dissolve precipitant

5. Enhance bacteriolysis by heating at 70-90℃ / 10min.
   **In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

6. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

7. After the process of disruption, add 200µL of SDS solution+ and enhance bacteriolysis at 70℃ / 10min.

8. Add 400µL of phenol mixture and shake on the mixer at 1min. Centrifuge at 15000rpm / 3min and collect 200µL of supernatant as crude DNA solution.

Purification of DNA/RNA

Purification is operated with Ethanol precipitation method or magnetic beads collection method

Purification with Ethanol Precipitation Method

1. Transfer 200µL of crude DNA solution to microcentrifuge tube (sterilized, 2mL capacity), add 1000µL of 99% Ethanol and mix with vortex. After treatment, centrifuge at 15000rpm / 3min and discard the supernatant

2. Add 1000µL of 70% Ethanol and centrifuge at 15000rpm / 3min. After this process, discard remaining supernatant alcohol sucking with micropipette (Please be careful not to suck in precipitate containing DNA/RNA).

*In case preparing self-made 70% Ethanol, add DEPC treated water to 99 % Ethanol and adjust 70% at w/v.

3. Add 200µL of DEPC treated water to and suspend the precipitate.

**Option : If concentration of DNA is too high, it may cause suppression of RNA or DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm/280nm, 260nm /230nm and choose appropriate amount for next step of gene amplification process.

I) Extraction of DNA/RNA from soil compost

Compositions of microflora in soil are differs by environment. Numbers of bacteria are various spanning from levels of 10^3 to 10^9.

Pre-treatment

Transfer 4g of soil sample to falcon tube (50mL capacity), add 35mL of buffer (125mM NaCl, 50mM EDTA, pH8.0) and 1mL of 20% skim milk. Suspend soil mixture with Vortex. (This treatment enables to remove inhibitory substance for PCR absorbed to Silica or DNA homogenizing samples).

After nixing, stay at 5-10min until supernatant of upper phase becomes clear. If this interval is to short, there may be a case process of phenol extraction may be operated more than twice.

Preparation of Soil Samples

1. Transfer 2mL of soil suspension to experimental tube, centrifuge at 15000rpm / 5min and discard the supernatant. Add more 2mL of same sample and repeat once more. After discarding the supernatant, add 150μL of lysis buffer, mix well and transfer whole suspension to tube containing beads.

2. Enhance bacteriolysis staying at 70-90℃ / 10min
   *In case extracting RNA, optimum temperature is 70℃. For DNA extraction, generally possible at 70℃ but if samples are derived from gram-positive bacteria, recommend temperature is 90℃ to improve disruption efficiency. Almost all of asporogenic pathogens are perished by this operation.

3. Close lid of the tube tightly, disrupt samples with appropriate device (Disrupter Genie : Scientific Industries). Even disruption device is not available, it is possible to extract DNA/RNA by treatment with Vortex at 2 min.

4. After the process of disruption, add 200μL of SDS solution$^+$ and enhance bacteriolysis at 70℃ / 10min.

5. Add 400μL of phenol mixture and shake on the mixer (or Vortex) at 1min.

6. Centrifuge at 15000rpm / 3min and transfer 300μL of the supernatant to sterilized microcentrifuge tube (in case using Epppendorf tube at 2ml capacity, alcohol is almost completely removed with the operation described in step 9).

7. Add 1mL of Ethanol, invert well and centrifuge at 15000rpm / 2min.

8. Discard the supernatant and add 1mL of 70% Ethanol. Invert well and centrifuge at 15000rpm / 2min.

9. Discard the supernatant carefully with pipette.

10. Dry up the DNA by air or centrifuge. Treat at 55℃/ 5min in case using Heat Block device.

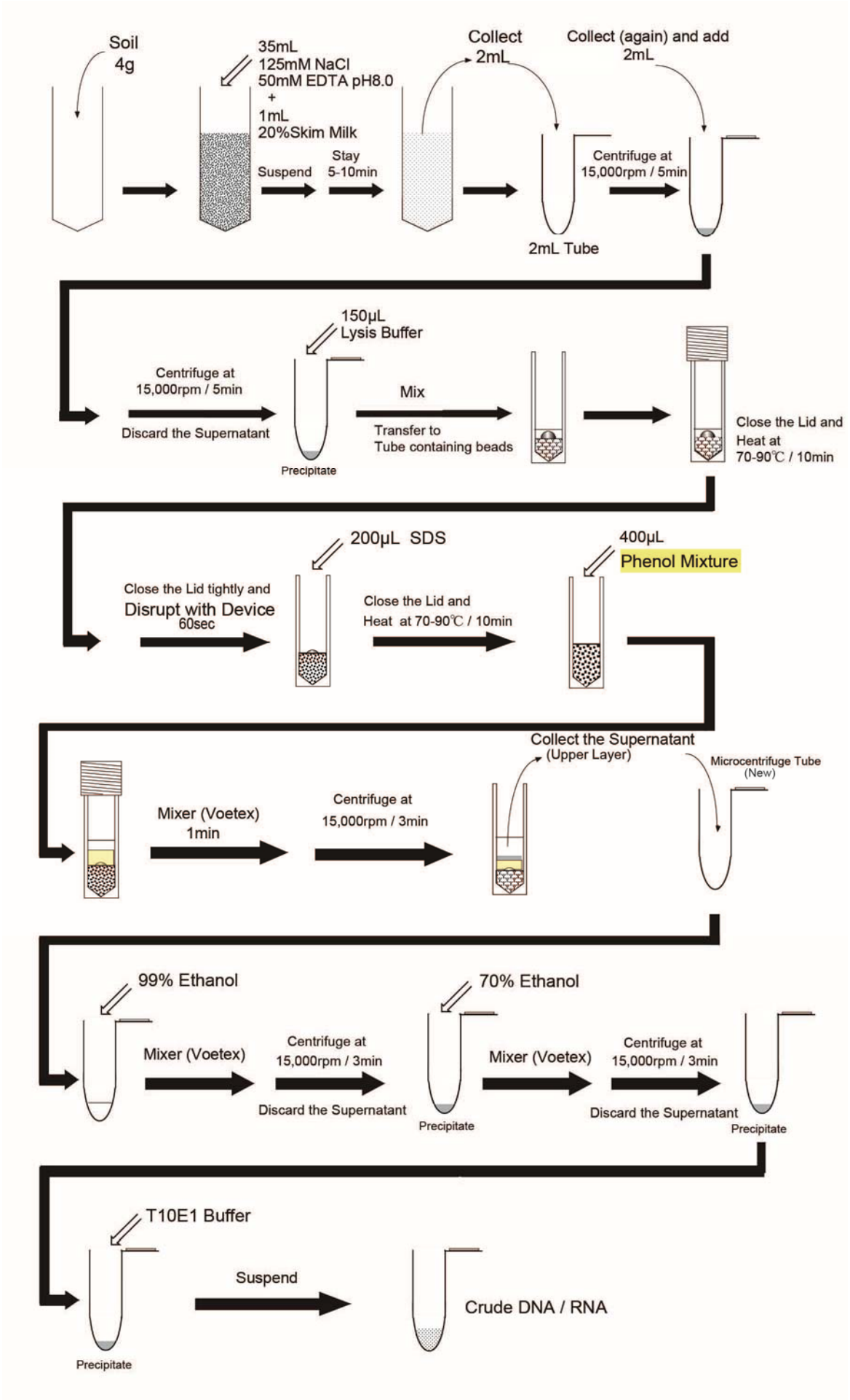
11. Add 200μL of T10E1 Buffer and use the mixture as crude DNA solution.

*Option

If concentration of DNA is too high, it may cause amplification of RNA or suppression of DNA amplification reaction. Record absorption value using NanoDrop at 260nm, 260nm / 280nm, 260nm / 230nm and choose appropriate amount for next step of gene amplification process.

(If absorption value is reach to the level of more than 1.8 at 260 / 280nm, it is possible to apply directory to PCR).

In this case, ahead to next step of "Purification example of crude DNA/RNA described next.



Example of purification from crude DNA / RNA solution

Purification of DNA is possible to operate using Silica-coated magnetic beads (commercially available).
DNA physically attaches to Silica while no absorption with Humic Acid. This difference of reactivity enables to isolate specifically DNA.

A) Example of DNA purification with magnetic Beads

1. Mix 200μL of crude DNA solution with 10μL of 5M NaCl and transfer to microcentrifuge tube.

2. Stay at 5min and capture the magnetic beads. Discard the supernatant after 1min of the treatment.

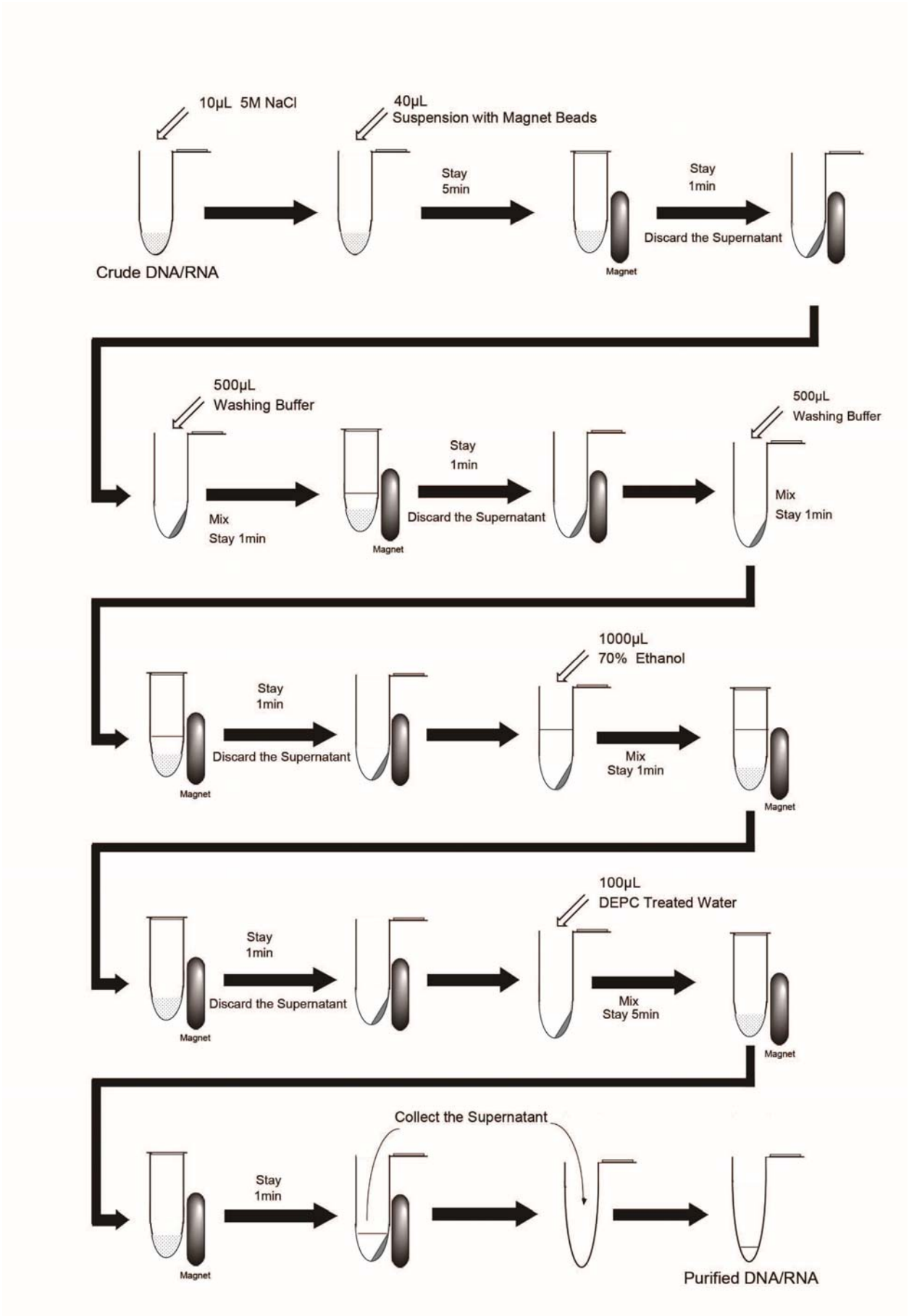
3. Add 500μL of wash buffer attached with beads, mix well and capture magnetic beads. Discard supernatant after 1 minute of the treatment.

4. Add 500μL of wash buffer again, mix well and capture the magnetic beads. Discard thesupernatant after 1min of the treatment.

5. Add 1000μL of 70% Ethanol washing solution, mix well and capture the magnetic beads. Discard the supernatant completely with care not to make the alcohol remain.

6. Add 100μL of DEPC treated water.

7. Stay at 5min /room temperature, capture the magnetic beads. Collect the supernatant after1min of the treatment and transfer to sterile tube.



7: Usage in gene amplification of extracted DNA by PCR

1) Storage of extracted DNA/RNA
   Cool the extracted DNA/RNA in ice immediately. In case it is not treated for gene amplification immediately, freeze the extracted DNA/RNA quickly.

2) Concentration of the extracted DNA
   When the extracted DNA is subjected to gene amplification, the high DNA level would inhibit PCR reaction. Therefore, it is recommended to adjust the concentration of the extracted DNA so that the amount of DNA can be kept less than 100 ng per a tube during amplification (about 25 ng/tube for the optimum amount). As a spectrophotometer is usually used to determine concentration/purity of DNA, the procedure is shown below.
   The concentration of nucleic acid is calculated according to the following equation, expressed as the concentration unit of μg/mL.

   [Absorbance at 260 nm (A260)] × [coefficient specific for the type of nucleic acid] × [optical path length of the cell (m)/10]

   When correction for optical path length is made by the software of the instrument, the last term is unnecessary.
   The coefficients for double strand DNA, single strand DNA or oligo DNA, and RNA are generally 50, 33, and 40, respectively.
   The purity test of the extracted nucleic acid is performed based on the "absorbance ratio" shown below.

   [Absorbance at 260 nm (A260)] / [Absorbance at 280 nm (A280)]

   As the value of A280 refers to the amount of protein or phenol contained in the sample, an ideal absorbance ratio is not less than 1.8. (When the value is significantly low, PCR may be inhibited. Therefore, it is recommended to repeat extraction and purification from addition of SDS solution^{+} (without heating) or to remove the contaminant of low molecules with such as spin columns.)

3) Buffer for storage
   It is preferable that the extracted/purified DNA/RNA be resuspended in DEPC-treated water and then immediately subjected to amplification. When it is stored, dissolve in RNase-free 10 mM Tris-1mM EDTA, pH 8.0 or pH 7.6 (TE buffer). Use the solution of around pH 8.0 and around pH 7.6 for DNA and RNA, respectively.

8: Precautions for use and handling

1) Precautions for handling

(1) During disruption with a homogenizer, the disruption conditions other than those with the recommended device (or a tabletop-type mixer) may damage the tube containing beads. Especially, vigorous shaking should be avoided in the device dedicated for cell-disruption with beads.

(2) Lysis buffer contains the substance that irritates the skin and may cause irritation on certain individuals. Wear gloves during operations.

(3) The phenol mixture contains phenol, which irritates the eyes, skin and mucosa, and chloroform, which irritates the eyes, nose, throat, and skin and may adversely affect the central nervous systems, liver, etc. Wear goggles, mask, and gloves and pay special attention when handle it.

(4) Take extra care so that the drug solutions should not contact with the skin, eyes, and mucosa or into the month. If it makes contact with skin, eyes, and mucosa, flush with running water immediately.

(5) Handle samples (specimens) after taking measures to prevent biohazard under the direction of the person with expertise in handling of microorganisms.

2) Precautions for use

(1) Not to use any expired product.

(2) Strictly observe the storage conditions.
Tubes containing beads, SDS solution^{+}, and DEPC-treated water can be stored at room temperature. Lysis buffer and phenol mixture must be stored under a light-resistant and refrigerated (2 to 10°C) condition.

(3) Not to use the product in which damage of the container or contamination is detected, if any.

(4) In particular, pay special attention to handling RNA. RNA is much more unstable and fragile than DNA; such as contamination of airborne dust into the tube or contact of hand with the inner side of the lid of the tube may cause degradation of RNA. Therefore, wear gloves and mask during experiment with RNA, use plastic or glass containers after removal of RNase, and keep the lid of the container or tube closed when not in use.

(5) Although less attention for decomposition enzyme is required for DNA than RNA, avoid contamination of substances such as bacteria other than the sample during operation.

3) Disposal Consideration

(1) The phenol mixture contains 42% of phenol and 40% of chloroform, and the lysis buffer contains 0.72% of 2-mercaptoethanol. Regardless of whether it is before or after use, dispose of these solutions according to the rules in the Waste Management and Public Cleaning Act, the Water Pollution Control Law, and the Poisonous and Deleterious Substances Control Law as well as instructions of the local authorities.

(2) In case any drug solution spills on the working table or floor, remove it immediately by wiping with such as waste cloth or in other ways followed by thorough cleaning such as a new waste cloth using water or alcohol (for phenol mixture). In case the beads drops, collect them as many as possible, and thoroughly wipe them up with such as wet waste cloth.

(3) Make sure to sterilize the tools used in treatment of specimens by heating in an autoclave (at 121°C for 30 minutes or longer), and take appropriate actions for disposal.

9: Storage
Light-resistant and refrigerated
Storage temperature: 2 – 10°C

10: Shelf life
9 months

Distibutor:
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7-8, Nihonbashi Kobuna-cho, Chuo-ku, Tokyo, 103-0024

Manufacturer:
AMR Advanced Microorganism Research
AMR Co., Ltd.
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