ABIOpure™ Extraction

ABIOpure™ Total DNA
(version 2.0)

Blood/Cell/Tissue Extraction Handbook

Cat No: M501DP100

FOR RESEARCH USE ONLY

AllianceBio

Table of Contents

Kit Components

Component	M501RP100	Storage Temp
No. of preps	100	
ABIOpure™ mini column type G	100	Room Temperature
Collection tube	300	Room Temperature
Buffer GP	25 ml	Room Temperature
Buffer YL	60 ml	Room Temperature
Buffer CL	25 ml	Room Temperature
Buffer BL	25 ml	Room Temperature
Buffer BW+	40 ml	Room Temperature
Buffer TW+	24 ml	Room Temperature
Buffer AE*	30 ml	Room Temperature
Proteinase K**	48 mg	Room Temperature
PK Storage Buffer	4 ml	Room Temperature

*10mM Tris-HCl, pH 9.0, 0.5mM EDTA

**After reconstitution of proteinase K, store it 4˚C or -20˚C

+Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle

Precautions

The buffers included in the ABIOpure™ DNA Blood/Cell Extraction kit contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Buffer BL contains chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid producing spills or aerosol. Any material coming in contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.
Never pipette solutions by mouth! Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid producing spills or aerosol. Waste must be handled and disposed of according to adequate safety measures. Disposable combustible material must be incinerated.

Stability & Storage

All components of the ABIOpure™ DNA Blood/Cell Extraction kit should be stored at room temperature (15 - 25˚C).
After reconstitution of Proteinase K with PK Storage Buffer, it should be stored at 4°C for preservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. However, for prolonged preservation of activity, storing at -20°C is recommended.
During shipment or storage under cold ambient condition, a precipitate can be formed in Buffer BL or CL. Heat the bottle at 56˚C to dissolve completely in such a case. Using precipitated buffers will lead to poor DNA recovery.
ABIOpureTM DNA Blood/Cell Extraction kits are guaranteed until the expiration date printed on the product box.

General Description

The ABIOpure™ Genomic DNA Blood/Cell kit provides fast and easy methods for the small scale purification of total DNA from various samples, such as blood, tissues, bacterial or cultured cells, and forensic specimens. Purified DNA can be used directly for PCR, Southern blotting and other downstream applications.
The ABIOpureTM Genomic DNA Blood/Cell Extraction kit utilizes the advanced silica-binding technology to purify total DNA sufficiently pure for many applications.

Various samples are lysed in optimized buffer containing detergents and lytic enzyme. Under high salt conditions, DNA in the lysate binds to a silica membrane and impurities pass through the membrane into a collection tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure DNA is released into a clean collection tube with deionized water or low ionic strength buffer.

Limitations

The ABIOpure™ Genomic DNA Blood/Cell kit is intended for Research Use Only applications.

Quality Control

All components of the ABIOpureTM Total DNA Blood/Cell Extraction kits are manufactured in a clean environment which is monitored periodically. To ensure product consistency and quality, the quality certification process is carried out on each lot of product.

Technical Support

If you need assistance, have any question or suggestion or if you experience any difficulties using ABIOpureTM extraction kits, please feel free to contact our technical support team at support@alliancebio.com.

Features

Format: Spin Column
Maximum amount of starting sample: 0.25 ml
Minimum amount of starting sample: 0.10 ml
Maximum loading volume: 700 µl
Minimum elution volume: 30 µl
Maximum binding capacity: 100 µg
Typical yield: 3 µg
Operation time: 30 minutes

Samples

ABIOpureTM Genomic DNA Blood/Cell Extraction kit can be used to extract DNA from fresh or frozen blood, body fluid, nucleated blood, lymphocyte, cultured cells, buccal swab, saliva, hair, sperm, fresh, frozen or fixed animal tissue, dried blood spot, gram-negative bacteria, gram positive bacteria, yeast and etc.

For long-term storage, it is recommended to store the sample lysate at -70°C (after proper mixing and homogenization with Buffer RB and β-mercaptoethanol).

Sample Amount and Expected Yield

ABIOpureTM Genomic DNA Blood/Cell Extraction kit is designed for preparation from a small amount of starting sample. The starting sample amount should not exceed the recommended maximum limit, otherwise DNA recovery will be significantly lowered. The recommended amount of starting sample and the yield is listed on the next page. For samples with a very high DNA content (e.g., buffy coat, spleen which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended should be used.

If your starting material is not listed or you have no information about your sample, we recommend you start with a smaller sample than the listed and increase the sample size in subsequent preparations depending on the result.

The DNA yield from whole blood will depend on the number of white blood cells (WBCs, leukocytes) contained in the sample. The number of WBCs varies from sample to sample, and can be determined using a hematocytometer or a cell counter. This kit can be used to extract total DNA from blood containing as little as 2.5 x 10^5 leukocytes per milliliter and up to 1 x 10^7 cells per milliliter.

Generally, the density of bacterial cells cannot be easily determined because its optical density is influenced by various factors, such as species, media and measuring devices. A rough guide may be helpful with the bacterial cells. A_{600}=1 corresponds to 1 x 10^9 cells per milliliter with *E.coli*. For yeast, A_{600}=1 is obtained with a cell density of 1 ~ 2 x 10^7 cells per milliliter.

Sample	Starting amount (max)	Yield (µg)
Whole blood	200 µl	12 ~ 3
Buffy coat	200 µl	40 ~ 20
Nucleated blood	10 µl	16 ~ 5
Cultured cells or lymphocytes	5×10^{7}	25 ~ 14
Brain	20 mg	18 ~ 5
Heart	20 mg	10 ~ 4
Kidney	20 mg	15 ~ 35
Liver	20 mg	35 ~ 15
Lung	20 mg	10 ~ 4
Pancreas	20 mg	25 ~ 8
Spleen	10 mg	35 ~ 10
Bacteria	2×10^{9}	25 ~ 5
Yeast	5×10^{7}	25 ~ 10

Table 1: The yield in this table is calculated by addition of each eluate of 3 successive elution steps after DNA preparation with RNase A treatment. Without RNase A treatment, average yield from some samples may be significantly different from this data.

Sample Preparation

The yield and purity of DNA can be varied depending on the methods for harvest-ing and/or storing the starting sample materials. Freshly harvested sample should be used or stored immediately for best results. Note that the sample should be placed on ice as quickly as possible and repeated freezing and thawing of frozen sample should be avoided.

Blood and Its Derivatives

Blood samples should be used or stored immediately after collected in tubes containing anticoagulants and preservatives for whole blood. Whole blood collected in anticoagulants, such as EDTA or citrates (CPDs and ACDs), can be stored for several days at 4°C and at least for 2 years at -80°C without significant change in its properties. EDTA, a metal chelator, is an inhibitor against metal-dependent nuclease and is the preferable anticoagulant for DNA preparation. Heparin can also be used as an anticoagulant but is not usually used because it acts as an inhibitor in the PCR reaction. Frozen blood should be thawed quickly in a 37°C water bath and kept on ice before use.

Blood derivatives, such as plasma, serum or buffy coat, can also be used for specific applications. Buffy coat can be used for higher yield of DNA and is prepared by collecting the intermediate phase after the centrifugation of whole blood.

150 - 250 µl of buffy coat can be collected from 3 ml of whole blood, but the concentration of leukocytes should be determined because overload of leukocytes will lead to poor results. If the number of leukocytes exceeds 5 x 10^6, DNA recovery will be significantly decreased.

Cultured Cells

Cultured cells growing in suspension can be easily harvested by centrifugation. However attached cells should be treated with trypsin-EDTA for detaching the cells before harvesting. The number of cells should be determined using a hematocytometer or other cell counter, and should not be over 5 x 10^6 per prep. Harvested cells washed with phosphate buffered saline (PBS) can be used directly in DNA preparation or stored at -20°C or -80°C in pellet. It is not recommended washing the fixed cells with PBS, because it can cause cell lysis and significant reduction in DNA yield. The sample should always be kept on ice before use.

Tissue

Harvested tissues should be used freshly or stored at very low temperature as quickly as possible. Generally, making the sample finer will lead to better results,

but will also reduce the experiment time. Grinding in mortal and pestle under liquid nitrogen is a good method for disrupting the sample, but alternative methods, such as a homogenizer or a bead-beater, can be employed in a case by case basis for efficient disruption. Shaking or vortexing during incubation for lysis may greatly accelerate the efficiency of lysis, resulting in reduced time for complete lysis. Note that the freshness and the particle size of the disrupted sample are key for good results. The frozen sample should be kept on ice until use.

Bacterial Cells

Bacterial cells can be prepared by incubating the culture for 12 ~ 24 hours at 37°C with vigorous shaking until the cells reach the log phase. Harvested bacterial cells can be used directly or stored at -20°C or -80°C for future use. Gram positive bacteria should be treated with lysozyme or lysostaphin to weaken the rigid and multilayered cell wall. Gram negative bacteria does not need this treatment. Extreme care should be taken with pathogenic bacteria.

Yeast Cells

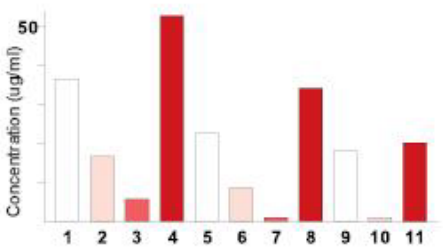
Yeast cells are troublesome for purification of DNA because the rigid cell wall does not lyse well using standard lytic conditions. The cell wall of yeast should be loosened by enzymatic lysis with a lytic enzyme such as lyticase or zymolase, and spheroplasts are then collected by centrifugation. The harvested spheroplasts can be used directly for DNA preparations or stored at -20°C or -80°C for later use. When the value of A600 is 1, the cell density of yeast culture may be 1 ~ 2 x 10^7 cells per milliliter.

Elution

Purified DNA is eluted from the ABIOpure™ mini column type G membrane in either deionized water or Buffer AE which contains 0.5 mM EDTA and 10 mM Tris-HCl, pH9.0. Elution buffer should be equilibrated to room temperature before applying to the ABIOpureTM mini column. Typically, elution is carried out in two successive steps using 200 µl Buffer AE each time. The volume of elution can be adjusted depending on the starting materials or the downstream applications,

but it should be greater than the minimum requirements to wet the entire column membrane (50 µl per column) and should not be over 300 µl. Basically, it is recommended, for the recovery of higher DNA concentration, to decrease the elution volume to a minimum, but total DNA recovery will decrease in this case. Otherwise, if maximum recovery is needed, the volume of elution buffer should be increased to elute as much as possible. Yield may be slightly increased if the mini column is incubated with the elution buffer at room temperature for 5min before centrifugation.

Generally, DNA bound to the mini column will not be eluted completely with a single elution step. Approximately 85 ~ 60 % of the DNA will elute in the first 200 µ l, and the rest of bound DNA in next 200 µl. However, a single elution with 200 µl of elution buffer will be sufficient to recover the amount of DNA required for multiple PCR reactions. For very small samples (containing less than 1 µg of DNA), only a single elution in 50 µl of buffer AE or deionized water is recommended. The mini column for ABIOpureTM Genomic DNA Blood/Cell Extraction co-purifies DNA and RNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, although it does not inhibit PCR itself. If RNA-free DNA is required, RNase A treatment should be used at the optional step included in each protocol. A treatment of RNase A will decrease the overall yield measured by spectrophotometer, but the virtual recovery of DNA will be slightly increased . Any RNase A at the concentration of 20 mg/ml can be used.



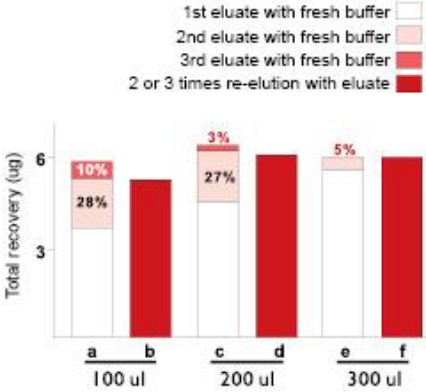


Fig. 2 The yield and concentration of purified DNA depending on the elution method.

DNA was prepared from 200 µl of bovine whole blood. Each preparation was exactly identical except the elution method: elution was performed 3 times per column with 100 µl (lane 3 ~ 1) and 200 µl (lane 7 ~ 5), and 2 times per column with 300 µl (lane 10 ~ 9) of fresh Buffer AE. At the same time, another elution was carried out 3 times (100 µl and 200 µl) and 2 times (300 µl) by recursive use of the eluate instead of fresh Buffer AE (lane 11 ,8 ,4). Total 11 eluates purified from 6 samples were resolved on 0.8 % agarose gel to visualize (upper left) and its concentration (lower left) and total yield (lower right) was measured by spectrophotometric analysis.

Materials required but not provided

- Laminar flow hood
- Pipette set (10µl, 100µl and 1000µl)
- RNA-free pipet tips
- Sterile 1.5 microcentrifuge tubes (nuclease-free)
- Suitable protection (ex. lab coat, disposable gloves, goggles, etc.)

Protocol for Blood and Body Fluids/Cultured Cells using Microcentrifuge

Before you start:

- Prepare the water bath to °56C
- Prepare absolute ethanol
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- If a precipitate has formed in Buffer BL, heat to dissolve at 56°C before use
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Pipet 20 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml tube.** If the sample volume is larger than 200 µl, increase the amount of Proteinase K proportionally. When the cell mass is low, up to 400 µl of starting sample can be used. For 400 µl of sample volume, 40 µl of Proteinase K solution is needed.

<table>
<tr>
<td>2.</td>
<td>Transfer 200 µl of sample to the tube. Use the starting sample listed below.
If the sample volume is less than 200 µl, adjust the volume to 200 µl with 1X PBS.
<table>
<tr><th>Sample</th><th>Max. amount per prep</th><th>Preparation</th></tr>
<tr><td>Mammalian whole blood</td><td>200 µl</td><td>Direct use</td></tr>
<tr><td>Body fluid*</td><td>200 µl</td><td>Direct use</td></tr>
<tr><td>Buffy coat</td><td>200 µl</td><td>Direct use</td></tr>
<tr><td>Nucleated blood of bird, fish, reptile and amphibian</td><td>200 µl</td><td>10 µl blood with 190 µl of 1X PBS</td></tr>
<tr><td>Cultured cells or lymphocyte</td><td>5 x 10^6</td><td>5 x 10^6 cells in 200 µl of 1X PBS</td></tr>
<tr><td>Virus*</td><td>200 µl</td><td>200 µl of virus containing media</td></tr>
</table>
</td>
</tr>
<tr>
<td>3.</td>
<td>Optional: If RNA-free DNA is required, add 20 µl of RNase A (20 mg/ml), vortex to mix and incubate 2 min at room temperature.
Unless an RNase A treatment is used, RNA will co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not PCR itself.</td>
</tr>
<tr>
<td>4.</td>
<td>Add 200 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at °56C for 10 min. Spin down briefly to remove any drops from the inside of the lid.
If the sample volume larger than 200 µl, increase the volume of Buffer BL in proportion. Ratio of Buffer BL to the starting sample volume is 1:1.</td>
</tr>
</table>

	It is essential to mix the sample and Buffer BL thoroughly for good results. Longer incubation will not affect DNA recovery.
5.	**Add 200 µl of absolute ethanol (not provided) to the sample. Pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from the inside the lid.** If the sample volume is larger than 200 µl, increase the ethanol volume proportionally.
6.	**Transfer the mixture to the mini column carefully, centrifuge for 1 min at 6000 x g (>8000 rpm) and replace the collection tube with the new one (provided).** If the starting sample is larger than 200 µl, apply the mixture twice: apply 700 µl of the mixture, spin down, discard the pass-through, re-insert the empty collection tube, and repeat this step again until all the mixture has been applied to the mini column. If the mixture has not passed completely through the membrane, centrifuge again a full speed (13,000 x g) until all the solution has passed through. Centrifugation at full speed is recommended to avoid clogging especially when applying samples with high cell density, such as buffy coat, lymphocyte or cultured cells. Centrifugation at full speed will not affect DNA recovery.
7.	**Add 600 µl of Buffer BW, centrifuge for 1 min at 6,000 x g (>8,000 RPM) and replace the collection with the new one (provided).** If the mini column has colored residue after centrifuge, repeat this step until no colored residue remains. See the Troubleshooting Guide for details. Centrifugation at full speed will not affect DNA recovery.

8.	**Apply 700 µl of Buffer TW. Centrifuge for 1 min at 6,000 x 6 (>8,000 RPM). Discard the pass-through and re-insert the mini column back into the collection tube.**
9.	**Centrifuge at full speed for 1 min to remove residual wash buffer. Place the mini column in a fresh 1.5 ml tube (not provided).** Care must be taken at this step for elimination the carryover of the Buffer TW. If a carryover of TW occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to a new 1.5 ml tube. Centrifugation must be performed at full speed (13,000 x g - 20,000 x g).
10.	**Add 200 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.** * For low cell-density samples, such as body fluids or virus, use 50 - 150 µl elution buffer based on the species and conditions of the starting sample and/or the downstream applications. Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of mini column membrane for optimal elution of DNA. Repeat of elution step with fresh 200 µl elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 µl of elution buffer will increase yields slightly. Each eluate can be separated in fresh tubes or can be collected to same tube, but more than 300 µl of eluate cannot be collected in a 1.5 ml tube because the mini column will come into contact with the eluate. If higher concentration of DNA is needed or starting sample amount is very small, a second elution can be carried out with the first eluate instead of fresh elution buffer. Alternatively for higher

	concentration, elution volume can be decreased to 50 µl. However the small volume of elution buffer will decrease the total yield of DNA recovery. For long-term storage, eluting in Buffer AE is recommended. However EDTA included in buffer AE can inhibit subsequent enzymatic reactions, so you can avoid such a problem by using distilled deionized water (> pH7.0) or Tris-HCl (>pH8.5). *When using water for elution, check the pH of water before elution.*

Protocol for Buccal Swab

Before you start:

- Prepare the water bath to °56C
- Prepare sterile sharp blade (or wire cutter) and tweezers
- Prepare 1X PBS and absolute ethanol
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- If a precipitate has formed in Buffer BL, heat to dissolve at °56C
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Scrape the swab firmly more than 5 - 6 times against the inside of cheek.** To avoid contamination from other materials, ensure that the person who provides the sample has not taken any food or drink in 30 min prior to sample collection.
2.	**Place the swab in a 2 ml sterilized microcentrifuge tube. Clip off handle of brush with sterile sharp blade or wire cutter. Add 400 µl of 1X PBS to the tube.**

2.	**Place the swab in a 2 ml sterilized microcentrifuge tube. Clip off handle of brush with sterile sharp blade or wire cutter. Add 400 µl of 1X PBS to the tube.** Cutters should be rinsed with 70% ethanol to prevent contamination between samples.
3.	**Optional: If RNA-free DNA is required, add 20 µl of RNase A (20 mg/ml), vortex to mix, and incubate 2 min at room temperature.** Unless an RNase A treatment is used, RNA will be co-purified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.
4.	**Apply 20 µl of Proteinase K (20 mg/ml) and 400 µl of Buffer BL to the sample. Vortex vigorously to mix immediately.** For efficient lysis, mix the sample completely.
5.	**Incubate at 56˚C for 10 min. Briefly centrifuge to remove any drops from the inside of the lid.**
6.	**Add 400 µl of absolute ethanol to the lysate, and mix well by vortexing. Briefly centrifuge to remove any drops from the inside of the lid.**
7.	**Transfer carefully up to 700 µl of the mixture to the mini column. Close the cap. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the mini column back into the collection tube.** Be careful not to wet the rim of the mini column.
8.	**Repeat step 7 until all the remaining mixture has been applied to the mini column. Replace the collection tube with new one (provided).**
9.	**Continue with step 7 in Blood and Body Fluid protocol (page 14).**

Protocol for Saliva and Mouthwash

Before you start:
- Prepare the water bath to °56C
- Prepare 1.5 ml microcentrifuge tube and 50 ml conical tube
- Prepare 1X PBS (Phosphate buffered saline) and absolute ethanol
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- If a precipitate has formed in Buffer BL, heat to dissolve at °56C
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Collect 10 ml of mouthwash in a 50 ml conical tube, or collect 1 ml of saliva by spitting in a 50 ml conical tube. If saliva is used, add 5 ml of 1X PBS to the sample and vortex to mix.** To avoid contamination from other materials, ensure that the person who provides the sample has not taken any food or drink in the 30 min prior to sample collection.
2.	**Centrifuge at 2,000 x g (3,000 rpm) for 5 min to pellet cells. Immediately and carefully decant the supernatant to prevent the loss of any cell pellets. Re-suspend completely the pellets in 200 µl of 1 x PBS.** If the pellets are loose, repeat centrifugation.
3.	**Optional: If RNA-free DNA is required, add 20 µl of RNase A (20 mg/ml), vortex to mix, and incubate 2 min at room temperature.** Unless an RNase A treatment is used, RNA will be co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4.	**Apply 20 µl of Proteinase K (20 mg/ml) and 200 µl of Buffer BL to the sample. Vortex vigorously to mix completely.** For efficient lysis, mix the sample completely.
5.	**Continue with step 4 in Blood and Body Fluid protocol (page 13).**

Protocol for Hair

Before you start:
- Prepare the water bath to 56˚C
- Prepare 1.5 ml microcentrifuge tube
- Prepare absolute ethanol
- Prepare Buffer H as follow: 10 mM Tris-HCl, pH10 ,8.0 mM EDTA,100 mM NaCl, %2 SDS, 40 mM DTT (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions)
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- If precipitate has formed in Buffer BL, heat to dissolve at 56˚C
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Collect hair sample in a 1.5 ml microcentrifuge tube.** The amount of starting sample should not exceed 30 mg. It is recommended to use 0.5 - 1 cm from the root ends of plucked hair samples.
2.	**Add 180 µl of prepared Buffer H and 20 µl of Proteinase K to the tube, and vortex to mix thoroughly.**

3.	**Incubate at 56˚C for at least 1 hour until the sample is dissolved. Spin down briefly to remove any drops from the inside of the lid.** Invert the tube occasionally to disperse the sample, or place it on a rocking platform. Hair follicles should be completely dissolved. However the hair shaft may be not dissolved completely and this residual solid material will not affect DNA recovery.
4.	**Continue with step 3 in Blood and Body Fluid protocol (page 13).**

Protocol for Sperm

Before you start:
- Prepare the water bath to 56˚C
- Prepare 1.5 ml microcentrifuge tube
- Prepare absolute ethanol
- Prepare buffer H2 as follow: 20 mM Tris-HCl, pH20 ,8.0 mM EDTA, 200 mM NaCl, 4% SDS, 80 mM DTT (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions)
- Equilibrate **B**uffer AE to room temperature
- All centrifugation should be performed at room temperature
- If precipitate has formed in Buffer BL, heat to dissolve at 56˚C
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Place 100 µl sperm in a 1.5 ml microcentrifuge tube. Add 100 µl of Buffer H2 and 20 µl of Proteinase K to the tube. Mix thoroughly by vortexing.**

2.	**Incubate at 56˚C until the sample is dissolved completely. Spin down briefly to remove any drops from the inside of the lid.** It may need at least 1 hour for complete lysis. Invert the tube occasionally to disperse the sample, or place it on a rocking platform.
3.	**Continue with step 3 in Blood and Body Fluid protocol (page 17).**

Protocol for Blood and Body Fluid Using Vacuum

Before you start:
- Prepare the water bath to 56˚C
- Prepare absolute ethanol
- Prepare 1.5 ml microcentrifuge tube
- Prepare vacuum system: manifold, trap, tubing & pump capable of 15-20 inch Hg
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- If a precipitate has formed in Buffer BL, heat to dissolve at 56˚C before use
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Pipet 20 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml tube.** If the sample volume is larger than 200 µl, increase the amount of Proteinase K proportionally. When the concentration of cells is low, up to 400 µl of starting sample can be used. For 400 µl of sample volume, 40 µl of Proteinase K solution is needed.

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<tr>
<td>2.</td>
<td>Transfer 200 µl of sample to the tube. Use the starting sample volume listed below.
If the sample volume is less than 200 µl, adjust the volume to 200 µl with 1X PBS.
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<tr><td>Virus</td><td>200 µl</td><td>200 µl of virus-containing media</td></tr>
</table>
</td>
</tr>
<tr>
<td>3.</td>
<td>Optional: If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml, Cat. No. 391-001) to the sample, pipet 2 - 3 times to mix and incubate for 2 min at room temperature.
Unless an RNase A treatment is used, RNA will be co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.</td>
</tr>
<tr>
<td>4.</td>
<td>Add 200 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 56˚C for 10 min. Spin down briefly to remove any drops from the inside of the lid.</td>
</tr>
</table>

	If the sample volume is larger than 200 µl, increase the volume of Buffer BL in proportion. Ratio of Buffer BL to the starting sample volume is 1:1. It is essential to mix the sample and **B**uffer BL thoroughly for good results. Longer incubation will not affect DNA recovery.
5.	**Add 200 µl of absolute ethanol (not provided) to the sample. Pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from the inside of the lid.** If the sample volume is larger than 200 µl, increase the ethanol volume proportionally.
6.	**Attach the mini column to a port of the vacuum manifold tightly. If available, use vacuum adaptors to avoid cross-contamination between the samples.** Most commercial vacuum manifold with luer connectors can be used with this protocol. If the mini column becomes clogged during this procedure, it is possible to switch to the procedure for purification by centrifugation (pages 13 - 14).
7.	**Transfer the mixture to the mini column by pipetting. Switch on the vacuum source to draw the solution through the mini column. When all liquid has been pulled through the mini column, release the vacuum.** If starting sample volume is larger than 200 µl, repeat this step until all of mixture have applied to the mini column. If the mixture has not passed completely through the membrane, you can switch to centrifugation protocol by step 6 at page 16.s 13-14.

8.	**Apply 600 µl of Buffer BW and switch on the vacuum source. When all liquid has been pulled through the mini column, release the vacuum.** If the mini column has colored residue after this step, repeat this step until no colored residue remain. See the Troubleshooting Guide for more details.
9.	**Apply 700 µl of Buffer TW and switch on the vacuum source. When all liquid has been pulled through the mini column, release the vacuum. Transfer the mini column into an empty collection tube (provided).**
10.	**Continue with step 9 in Blood and Body Fluid protocol (page 14).**

Before you start:
- Prepare the water baths or incubators to 56˚C and 70˚C
- Prepare absolute ethanol
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers BL and CL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath.
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Homogenize up to 20 mg of tissue as described in step 1a, 1b or 1c, depending on the sample type.**

	To disrupt the sample finer will accelerate lysis and decrease the lysis time. For spleen tissue, up to 10 mg can be processed.
	1a. For soft tissue, such as liver or brain, put up to 20 mg of the tissue into 1.5 ml tube, add 200 µl of Buffer CL, and homogenize thoroughly with a microhomogenizer. Carefully homogenize for minimization of foaming
	1b. If a microhomogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 ml tube. Add 200 µl of Buffer CL and pulse-vortex for 15 sec.
	1c. If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put the tissue into a 1.5 ml tube. **Add 200 µl of Buffer CL and pulse-vortex for 15 sec.**
	******Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.***
2.	**Add 20 µl of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate at 56˚C until the sample is completely lysed. Spin down the tube briefly to remove any drops from the inside of the lid.** It is essential to mix the components completely for proper lysis. Lysis time varies from 10 min to 3 hours usually depending on the type of tissue and the homogenization method (step 1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation. If the sample is lysed in a water bath or heating block, vortex occasionally (2 - 3 times per hour) during incubation to lyse

	readily. Lysis in a shaking water bath, shaking incubator or agitator would be best for efficient lysis.
3.	**Optional: If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml), vortex to mix thoroughly, and incubate for 2 min at room temperature.** Unless RNase A treatment is used, RNA will be co-purified with DNA, especially when using transcriptionally active tissues, such as liver and kidney. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.
4.	**Add 200 µl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 70˚C for 10 min. Spin down briefly to remove any drops from the inside of the lid.** Cool down to room temperature before proceeding. It is important to mix the sample and buffer BL thoroughly for good result.
5.	**Add 200 µl of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from the inside of the lid.** It is important to mix the sample and ethanol completely for good results. After addition of ethanol, a white precipitate may be formed. It is essential to apply all of the mixture including the precipitate to the mini column in the next step.
6.	**Transfer all of the mixture to the mini column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).** If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 x g) until all of the solution

	has passed through. Centrifugation at full speed will not affect DNA recovery.
7.	**Add 600 µl of Buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with a new one (provided).** If the mini column has colored residue after centrifuge, repeat this step until no colored residue remains. See the Troubleshooting Guide for more details. Centrifugation at full speed (>13,000 x g) will not affect DNA recovery.
8.	**Apply 700 µl of Buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the mini column back into the collection tube.** Centrifugation at full speed will not affect DNA recovery.
9.	**Centrifuge at full speed (>13,000 x g) for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml tube (not provided).** Care must be taken at this step for eliminating the carryover of **B**uffer TW. If a carryover of **B**uffer TW occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to the new 1.5 ml tube. Centrifugation must be performed at full speed (13,000 x g - 20,000 x g).
10.	**Add 200 µl of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed (>13,000 xg) for 1 min.**

	***** *For a sample expected to yield little DNA, such as paraffin-embedded, formalin-fixed tissue, or dried blood spot or sperm, it is recommended to use 50 - 150 µl elution buffer, based on the species and conditions of the starting sample or the downstream applications.* Ensure that the Buffer AE or sterilized water is dispensed directly into the center of mini column membrane for optimal elution of DNA. Repeat of the elution step with fresh 200 µl elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 µl of elution buffer will only increase yield slightly. Each eluate can be separated in fresh tubes or can be collected to same tube, but more than 300 µl of eluate cannot be collected in a 1.5 ml tube because the mini column will come into contact with the eluate. If higher concentration of DNA is needed or the starting sample amount is very small, the second elution can be carried out with the first eluate instead of fresh elution buffer. Alternatively for higher concentration, the elution volume can be decreased to 50 µl. However the small volume of elution buffer will reduce the total yield of DNA recovery. For long-term storage, eluting in Buffer AE is recommended. But EDTA included in Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid this problem by using distilled deionized water (>pH7.0) or Tris-HCl (>pH8.5). When using water for elution, check the pH of water before elution.

Protocol for Paraffin-Fixed Tissue

Before you start:
- Prepare xylene and absolute ethanol
Xylene is an irritant and appropriate precautions should be taken in handling. For example, wear gloves, safety goggles, and a laboratory coat. Avoid contact with skin, eyes and clothing and work under a fume hood.
- Prepare the water bath to 56˚C
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath.
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Place a small section of paraffin-fixed tissue (up to 25 mg) in a 2 ml microcentrifuge tube.** Minced tissue may be de-paraffinized more efficiently.
2.	**Add 1200 µl xylene. Vortex vigorously until the paraffin has been completely melted. Centrifuge at full speed (>13,000 x g) for 5 min. Carefully remove supernatant by pipetting.** Be careful not to lose any of the pellet.
3.	**Add 1200 µl of absolute ethanol to the pellet to remove residual xylene and mix by vortexing.**
4.	**Centrifuge at full speed for 5 min. Carefully remove the ethanol by pipetting.** Do not remove any of the pellet.

5.	**Repeat the steps 3 - 4 once or twice.**
6.	**Evaporate the residual ethanol by incubating the microcentrifuge tube at room temperature for 10 - 15 min with an opened cap.**
7.	**Apply 180 µl of Buffer CL and mix completely by vigorous vortexing. Continue with step 2 of Tissue protocol on pages 24-25.**

Protocol for Alcohol-Fixed or Formalin-Fixed Tissue

Before you start:
- Prepare absolute ethanol
- Prepare the water bath to 56˚C
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Briefly blot excess fixative from tissue on clean absorbent paper. Place a small section of fixed tissue (up to 20 mg) in a 1.5 ml tube.** Minced tissue may be lysed more efficiently.
2.	**Apply 400 µl of 1X PBS to the tube. Vortex to mix, and briefly centrifuge to pellet tissue. Carefully remove supernatant.**

	Remove supernatant by pipetting not to lose the tissue.
3.	**Repeat step 2 once or twice.**
4.	**Add 180 µl of Buffer CL. Continue with step 2 of Tissue protocol (pages 23 -24).**

Protocol for Dried Blood Spot

Before you start:
- Prepare absolute ethanol
- Prepare water baths or incubators to 56˚C, 70˚C and 85˚C
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath.
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

** This protocol is suitable for blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper. (Schleicher and Schuell 903 or any equivalent.)*

1.	**Place 3 - 4 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 200 µl of Buffer CL.** Use a 3 mm (8/1”) single-hole paper puncher to cut out the circles from a dried blood spot.
2.	**Incubate at 85˚C for 10 min. Spin down briefly to remove any drops from inside of the lid.** Do not incubate for more than 15 min.

3.	**Add 20 µl of Proteinase K, vortex to mix, and incubate at 56˚C for 1 hour. Spin down briefly to remove any drops from the inside of the lid.**
4.	**Add 200 µl of Buffer BL and mix thoroughly by vortexing. Incubate at 70˚C for 10 min. Spin down briefly to remove any drops from the inside of the lid.** It is essential to mix the sample with Buffer BL completely for efficient lysis. After addition of Buffer BL, a white precipitate may be formed. This precipitate may be dissolved during incubation at 70˚C and will not affect DNA recovery.
5.	**Continue with step 5 of Tissue protocol on page 25.**

Protocol for Gram Negative Bacteria

Before you start:
- Prepare water baths or incubators to 56˚C and 70˚C
- Prepare 1.5 ml microcentrifuge tube
- Prepare absolute ethanol
- Equilibrate buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Harvest cells (up to 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube by centrifugation for 1 min at full speed. Discard supernatant.** 1 - 2 ml of overnight bacterial culture (A_{600}=1) may correspond to 1 - 2 x 10^9 cells.
2.	**Re-suspend completely the cell pellet in 200 µl of Buffer CL.**
3.	**Pipet 20 µl of Proteinase K solution (20 mg/ml). Vortex vigorously to mix completely. Incubate at 56˚C for 15 min.** After complete lysis, lysis mixture will turn to clear from turbid. If the lysate still looks turbid or cloudy, incubate until the lysate become clear without any particle. Lysis time may vary depending on the species and cell numbers. Cells can be further incubated for complete lysis and longer incubation time does not affect the yield. After incubation, cool the lysate to room temperature.
4.	**Spin down the tube briefly to remove any drops from the inside of the lid.**
5.	**Continue with step 3 of Tissue protocol on page 25.**

Protocol for Gram Positive Bacteria

Before you start:

- Prepare water baths or heating blocks to 37˚C, 56˚C and 70˚C
- Prepare Lysozyme (LYS702, Bioshop, Canada, or equivalent) or Lysostaphin (L7386, SIGMA, USA, or equivalent)
- Prepare 1.5 ml microcentrifuge tube
- Prepare absolute ethanol

- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 56˚C water bath.
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

Prepare Enzyme Mixture: Re-suspend the appropriate enzyme (not provided, listed below) with Buffer GP just before use. Enzyme mixture should be stored at -20˚C (or below) in small aliquots, ideally, one thaw per an aliquot. Thawed aliquot should be discarded.

30 mg/mL lysozyme (LYS702, Bioshop, Canada, or equivalent)
or/and
300 ug/mL lysostaphin (L7386, SIGMA, USA, or equivalent)

* For certain species, such as *Staphylococcus*, treatment of lysostaphin (final conc.=300 ug/ml) may be required for efficient lysis instead of (or with) lysozyme. However, lysozyme is sufficient to lyse the cell wall for most gram positive bacterial strains.

1.	**Pellet cells (~ 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube by centrifugation for 1 min at full speed. Discard the supernatant.**
2.	**Re-suspend the pellet thoroughly in 180 µl of the prepared enzyme mixture. Incubate at 37˚C for 30 min.** The purpose of this treatment is to weaken the cell wall so that efficient cell lysis can take place.
3.	**Optional:** If RNA-free DNA is required, add 20 µl of an RNase A solution (20 mg/ml) to the sample, mix well by vortexing and incubate for 2 min at room temperature. Unless an RNase A treatment is used, RNA will be co-purified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4.	**Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer BL. Mix completely by vigorous vortexing or pipetting.**
5.	**Incubate at 56˚C for 30 min and then for a further 30 min at 70˚C.** If any pathogen is suspected, it is strongly recommended that additional incubation at 70˚C for 30 min should be substituted by incubation at 95˚C for 15 min. Longer incubation at 95˚C will degrade DNA. After incubation, cool to room temperature.
6.	**Spin down the tube briefly to remove any drops from the inside of the lid.**
7.	**Continue with step 5 of Tissue protocol on page 25.**

Protocol for Yeast

Before you start:
- Prepare water baths or incubators to 37˚C and 56˚C
- Prepare the enzyme for lysing the cell wall. lyticase or zymolase
- Prepare 1.5 ml microcentrifuge tube
- Prepare absolute ethanol
- Equilibrate Buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffers CL and BL may precipitate at cool ambient temperature. If so, dissolve the buffer in a 37˚C water bath.
- *Before first use, add absolute ethanol (ACS grade or better) into buffers BW and TW as indicated on the bottle*

1.	**Add 3 ml (up to 5 x 10^7 cells) of a culture grown in YPD broth to a 15 ml centrifuge tube. Centrifuge at 5,000 x g for 10 min to pellet the cells. Remove the supernatant.** Alternatively, harvest twice in 1.5 ml or 2 ml micro-centrifuge tube. If a 1.5 ml or 2 ml tube is employed, centrifuge at full speed (>13,000 x g) for 1 min, discard the supernatant and repeat again with the remaining culture. When the value of A_{600} reaches to 1.0 (generally, log-phase), 3 ml of culture may yield approximately 25 ~ 10 ug of DNA.
2.	**Re-suspend the pellet thoroughly in 500 µl of Buffer YL.**
3.	**Add 200 U of lyticase (not provided) or 20 U of zymolase (not provided) and gently pipet to mix completely.** The unit/mg of lyticase varies depending on the manufacturer.
4.	**Incubate at 37˚C for 30 - 60 min to digest the cell wall.** Incubated cells turn to spheroplasts at this step, and this makes the lysis easier.
5.	**Centrifuge at 5,000 x g for 5 min. Remove the supernatant.** If 1.5 ml centrifugation tube is used, centrifuge at full speed for 1 min.
6.	**Re-suspend completely the cell pellet in 200 µl of Buffer CL.**
7.	**Pipet 20 µl of Proteinase K solution (20 mg/ml). Vortex vigorously to mix completely. Incubate 56˚C for 15 min.** After complete lysis, the lysis mixture will turn to clear from turbid. If the lysate still looks turbid or cloudy, incubate until the lysate become clear without any particles. Lysis time may vary depending on the species and cell numbers.

	Lysis time may vary depending on the species and cell numbers. Cells can be further incubated for complete lysis. The longer incubation time does not affect the yield. After incubation, cool the lysate to room temperature.
8.	**Spin down the tube briefly to remove any drops from the inside of the lid.**
9.	**Continue with step 3 of Tissue protocol on page 25.**

Troubleshooting

Problem	Possible Causes	Possible Reasons/ Solution
Low or no recovery	Too few cells in the starting sample	Some samples may have low concentration of cells, and some whole blood may contain low concentration of white blood cells. Increase the sample volumes and load the mini column several times. Reduce the elution volume to minimum. When the cell mass is low, it is also recommended to use carrier NA (e.g. Poly-dN, glycogen, or tRNA dissolved at 40 ~ 20 ug/ml in buffer BL).
	Too many cells in the starting sample	Sample amount over the maximum capacity will lead to poor lysis, resulting in significantly low recovery. Reduce the amount of starting sample or increase the volume of buffers proportionally.
	Inefficient or insufficient lysis	Inefficient lysis may be due to several causes: - Insufficient mixing with buffer BL - Too much cells in the starting sample

		- Degenerated Proteinase K - Poor disruption of tissue After addition of buffer BL in protocol, vortex the mixture vigorously and immediately to mix completely. If too much cells present in the sample, reduce the starting sample volume, or increase the volume of buffer BL to double. Using tissue as sample material, lysis should be continued until the tissue is completely lysed. Completely lysed sample will not have any particulate in lysate. Proteinase K should be stored under 4˚C for maintenance of proper activity. However, it is recommended to store in small aliquots at -20˚C for prolonged preservation of its activity.
	Improper eluent	As user's need, elution buffer other than buffer AE can be used. However, the condition of optimal elution should be low salt concentration with alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that condition.
Mini column has colored residue on the column membrane	Insufficient lysis	Insufficient lysis may result in colored residue remains on the MINI column membrane. Repeat the procedure after consideration of 'Inefficient lysis' at "Low or no recovery"
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with buffer BW.

Column clogging	Inefficient lysis	Insufficient lysis cause low DNA purity, and is due to insufficient mixing with buffer BL, too much cells in the starting sample, or degenerated Proteinase K. Check these out on next preparations.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with buffer BW before washing with buffer TW.
High A280/260 ratio	RNA contamination	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out RNase A treatment on protocol optionally.
Low A280/260 ratio	Insufficient lysis	Insufficient lysis cause low DNA purity, and is due to insufficient mixing with buffer BL, too much cells in the starting sample, or degenerated Proteinase K. Check these out on next preparations.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with buffer BW before washing with buffer TW.
Low concentration of DNA in eluate	Low cells in starting sample (too high elution volume)	Increase the volume of starting sample with additional volume of buffer used, and/or reduce the elution volume to 50 µl or do re-elution with eluate. For higher concentration of DNA in eluate, refer to the 'Elution' section of General considerations at page 12.

Degraded DNA	Starting sample is too old or mis-stored	Too old or improperly stored sample often yield degraded DNA. Use fresh sample.
DNA floats out of well while loading of agarose gel	Residual ethanol from Buffer TW remains in eluate	Ensure that the TW wash step in protocol has been performed correctly. Mini column membrane should be completely dried via additional centrifugation or air-drying. Refer the annotation of TW washing step.
Enzymatic reaction does not perform well with purified DNA	Low purity of DNA	Check "Low A280/260 ratio".
	RNA contamination	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, perform RNase A treatment step optionally.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with buffer BW.
	High salt concentration in eluate	Ensure that all washing steps were performed just in accordance with the protocols. Alternatively, carry out additional washing step with buffer TW. It may help remove high salt in eluate.
Precipitate in Buffer BL or CL	Buffer stored in cool ambient condition	For proper DNA purification, any precipitate in buffer BL/CL should be dissolved by incubating the buffer at 37˚C or above until it disappears.

Ordering Information

Product Name	Cat. No.	# of Preps
ABIOpure™ Total DNA Blood/Tissue/Cell	M501DP100	100 preps
ABIOpure™ Total RNA Cell-Free Fluids	M541RP50-A	50 preps
ABIOpure™ Total RNA Blood	M541RP50-B	50 preps
ABIOpure™ Viral DNA/RNA	M561VT50	50 preps

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