Nuclefy
Genomic DNA Purification Kit

Spin Column-Based Nucleic Acid Extraction

Instruction Manual

Version 1.1

trax

LAB SYSTEMS

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1. INTRODUCTION

The Nuclefy Genomic DNA Purification Kit is designed for rapid and reliable sample lysis, RNA removal and purification of intact genomic DNA (gDNA) from a variety of biological sample types. The protocol utilizes specifically formulated buffer systems allowing to process blood, tissues, and cultured cells. Supplemental protocols allow to process clinically-relevant samples such as saliva and buccal swabs, as well as bacteria, yeast and insects. In addition, it can also be used for classic gDNA cleanup.

The purified gDNA has high quality metrics, including A260/280 > 1.8 and A260/230 > 2.0, high DIN scores and minimal residual RNA. The purified gDNA is suitable for numerous downstream applications such as qPCR, end-point PCR, SNP analysis, genotyping, microarray analysis and library preparation for next generation sequencing.

2. KIT COMPONENTS

Component	150 Preps	Storage condition
Nuclefy Purification Columns	3 x 50 pcs	+15 to +25 °C
Nuclefy 2 ml Collection Tubes	6 x 50 pcs	+15 to +25 °C
Nuclefy Blood Lysis Buffer	15 ml	+15 to +25 °C
Nuclefy Cell Lysis Buffer	15 ml	+15 to +25 °C
Nuclefy Tissue Lysis Buffer	30 ml	+15 to +25 °C
Nuclefy Binding Buffer	60 ml	+15 to +25 °C
Nuclefy Wash Buffer	50 ml	+15 to +25 °C
Nuclefy Elution Buffer	30 ml	+15 to +25 °C
Nuclefy Proteinase K	1.6 ml	-15 to -25 °C upon arrival
Nuclefy RNase A	450 µl	-15 to -25 °C upon arrival

Table 1. Nuclefy Genomic DNA Purification Kit content.

3. STORAGE AND STABILITY

Nuclefy Proteinase K and Nuclefy RNase A enzymes must be stored at -15 to -25 °C upon arrival. All other kit components can be stored at room temperature (+15 to +25 °C). Always keep bottles tightly closed and columns as well as collection tubes sealed in the enclosed zip-lock bag. Lysis Buffers and Wash Buffer may form precipitates when stored under cool conditions. Check buffers for precipitate before use and re-dissolve at +37 °C if necessary. The expiry dates of the kit and single components are stated on the outer packaging as well as on the individual reagents.

4. PRECAUTION

Proper laboratory safety practices should be employed, including the use of lab coat, gloves and protective goggles. For information regarding the composition of the buffers, please consult the Safety Data Sheets (available upon request).

5. PROCEDURE OVERVIEW

Sample Lysis

Three specifically formulated Lysis Buffers are available, which meet the requirements for optimal processing of various sample types. Their main differences are:

- **Blood Lysis Buffer:** Rapid degradation of protein components including hemoglobin, in combination with protective property against a high nuclease activity.
- **Tissue Lysis Buffer:** Optimized for rapid digestion of all common tissue types.
- **Cell Lysis Buffer:** Allows to overcome viscosity commonly present in cell samples.

RNA Removal

The buffer systems are developed for optimal and selective binding of gDNA. To counteract the inevitable co-isolation of RNA, an optional RNase A digestion can be performed. Approximate RNA content without RNase treatment: 1 % for blood, 1-4 % for tissue, up to 10 % for cells.

Binding and Washing

A chaotrophic salt-based Binding Buffer is used for the selective binding of the gDNA with minimal RNA binding capability. To ensure maximal recovery/efficiency, binding to column's silica membrane takes place with sequential - low speed followed by maximum speed - spin steps.

6. ILLUSTRATED PROTOCOL

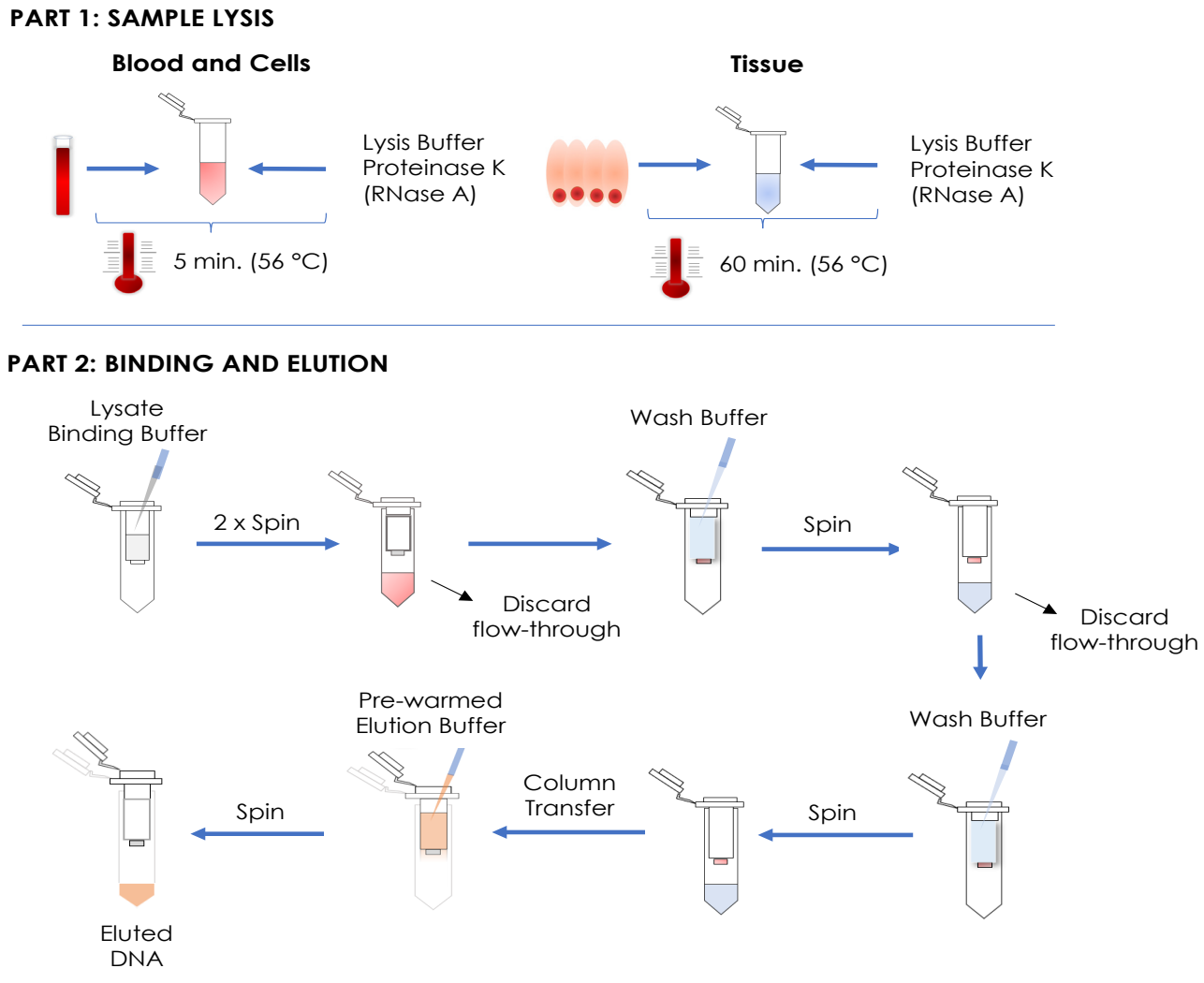

Figure 1. Illustrated gDNA purification protocol.

7. ELUTION AND STORAGE

Buffer Composition
The Elution Buffer is 10 mM Tris-HCl, 0.1 mM EDTA, pH 9.0. This composition favors inactivation of nucleases and gDNA long term storage. Alternatively, low salt buffers or nuclease-free water can be used.

Temperature
Elution is strongly recommended with Elution Buffer pre-heated to +60 °C to improve elution efficiency. Especially larger DNA molecules tend to bind more tightly to the silica membrane with pre-heated buffer.

Volume
The recommended elution volume ranges between 35-100 µl. A higher elution volume results in higher yield, but decreased gDNA concentration. With lower volume, a higher overall fraction of Elution Buffer may retain in the membrane. Thus, the concentration does not linearly correlate with the elution volume.

Exemplary, a human whole blood sample was processed. Elution volumes of 100, 75, 50, 40, 35, 30 and 25 µl were used on triplicate samples. The gDNA concentration measurement results were confirmed by qPCR detecting a well established target for gDNA yield determination:

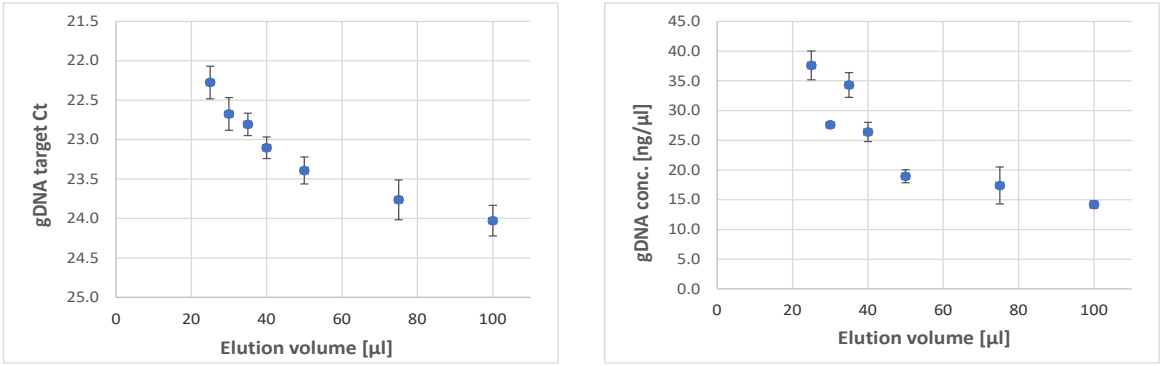

Figure 2. Determination of genomic DNA recovery following various elution volumes with qPCR and concentration measurement.

Efficiency
In addition to the initial elution step, a second elution step can be performed that may increase the overall yield, but decreases the DNA concentration. If a high DNA concentration is required, repetition of the elution step using the first eluate may increase yield while maintaining elution volume.

Storage
The eluted gDNA can be safely stored at +4 to +8 °C for weeks to months. For long term storage, store eluted gDNA at -80 °C. Repeated freeze-thaw processes should be avoided to maintain eluate integrity.

8. ADDITIONAL MATERIAL REQUIRED

General

- Ethanol, absolute
- Suitable reagent dispensing options
- Vortexer
- Thermal mixer for 1.5 ml microcentrifuge tubes (or an alternative compatible heating device)
- Water bath (or an alternative heating device to pre-heat buffers)
- Tissue homogenizer (or an alternative tool to cut e.g. tissue into small pieces)

Specific

Several protocols require the availability of additional non-supplemented material and chemicals (check the Pre-Application Preparation section, page 12).

9. SPECIFICATIONS

Sample input	Mammalian whole blood: 100 µl Nucleated red blood cells: 10 µl Tissue: up to 25 mg (depending on the tissue type) Insects: up to 20 mg Bacteria: up to 2 x 10^9 cells Yeast: up to 5 x 10^7 cells Saliva: up to 500 µl Cultured cells: up to 5 x 10^6 cells Genomic DNA-requiring cleanup
Binding Capacity	14-25 µg
Yield	Varies depending on sample type
Genomic DNA Size	Peak size > 50 kb for most sample types; may be lower for saliva and buccal swab samples
RNA Content	< 1 % (with included RNase A treatment)
Purity	A260/280 > 1.8 A260/230 > 2.0

Table 2. Kit Specifications.

10. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The Nuclefy Genomic DNA Purification Kit generates excellent input material regarding DNA integrity and purity for downstream applications such as quantitative PCR and NGS library preparation.

DNA Integrity

Genomic DNA samples were analyzed regarding DNA integrity by loading 50 ng on a 1 % agarose gel. Genomic DNA purification was performed following the appropriate purification protocols. Three representative DNA obtained from tissue (chicken heart, lung and muscle), human whole blood, cattle blood, HeLa and NIH/3T3 cells, *E.coli*, *R. terrae*, saliva as well as buccal swab are shown.

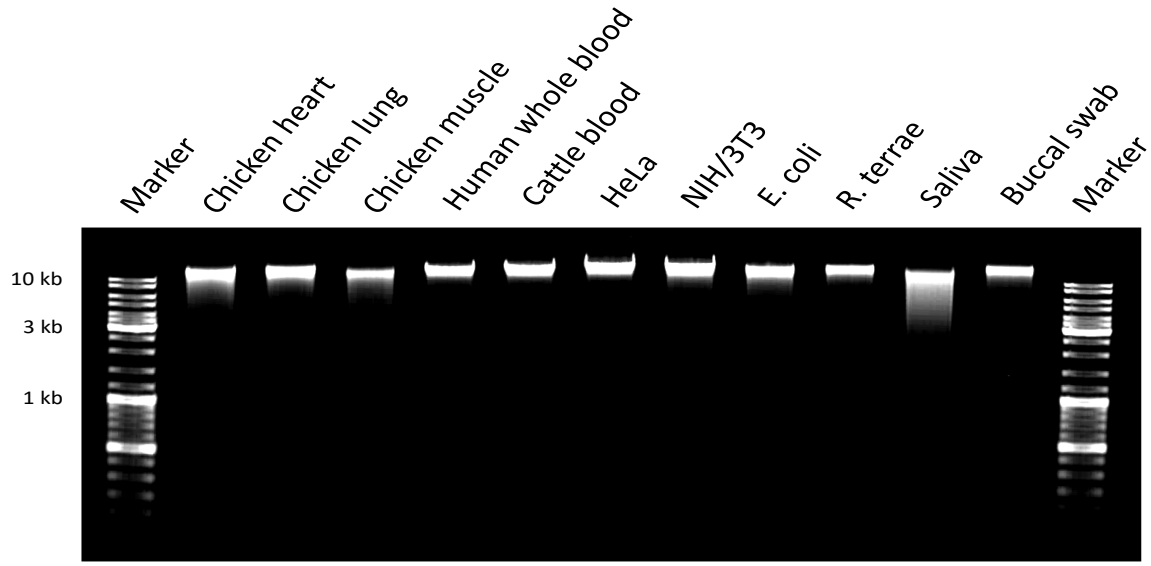

Figure 3. Integrity of gDNA isolated from representative sample types by agarose gel electrophoresis.

Quantitative PCR

Purified gDNA from several representative sample types was diluted to produce a four log range of input template concentrations. Results were obtained using primers targeting poultry- (chicken muscle), human- (whole blood, buccal swab, HeLa cells) as well as bacteria-specific (*E. coli*) genomic sequences for qPCR assays with the PhoenixDx® qPCR Mastermixes (Procomcure Biotech #PCCSKU12011) and cycled on Applied Biosystems™ QuantStudio 5 qPCR thermal cycler. Results confirm that eluted gDNA is highly pure and free from inhibitors, optimal for qPCR.

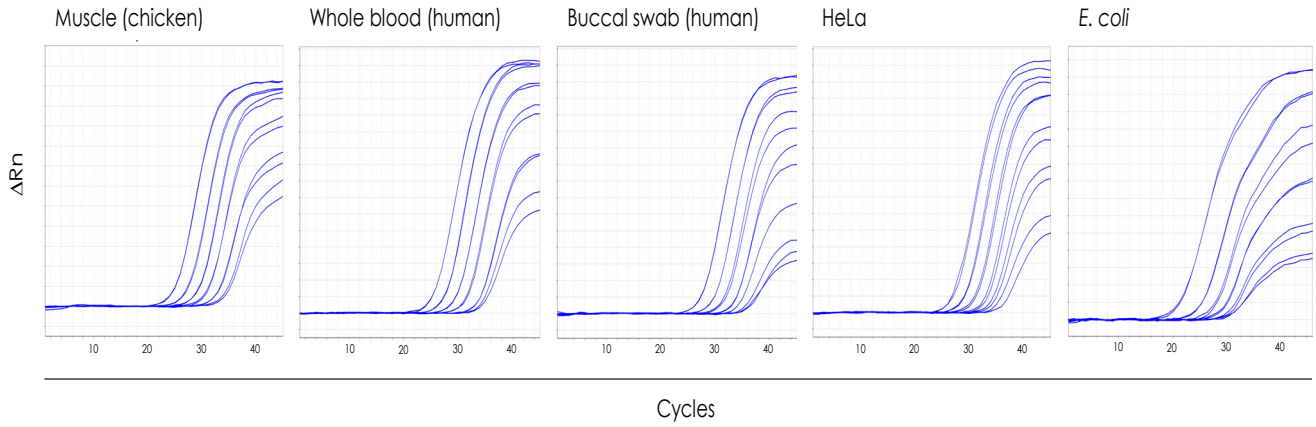

Figure 4. qPCR data using species-specific designed assays.

NGS Library Preparation

Genomic DNA was isolated from whole human blood with Nuclefy Genomic DNA Purification Kit (Procomcure Biotech #PCCSKU16073) and Monarch® Genomic DNA Purification Kit (NEB #T3010). Duplicate libraries were prepared from 200 ng human genomic DNA using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB #E7805). Libraries were sequenced on an Illumina MiSeq®. Reads were mapped using BWA and GC coverage was calculated using Picard's CollectGCBiasMetrics (v2.26.2). Library yields of samples were assessed on a Caliper Labchip GX II using a High Sensitivity DNA Kit.

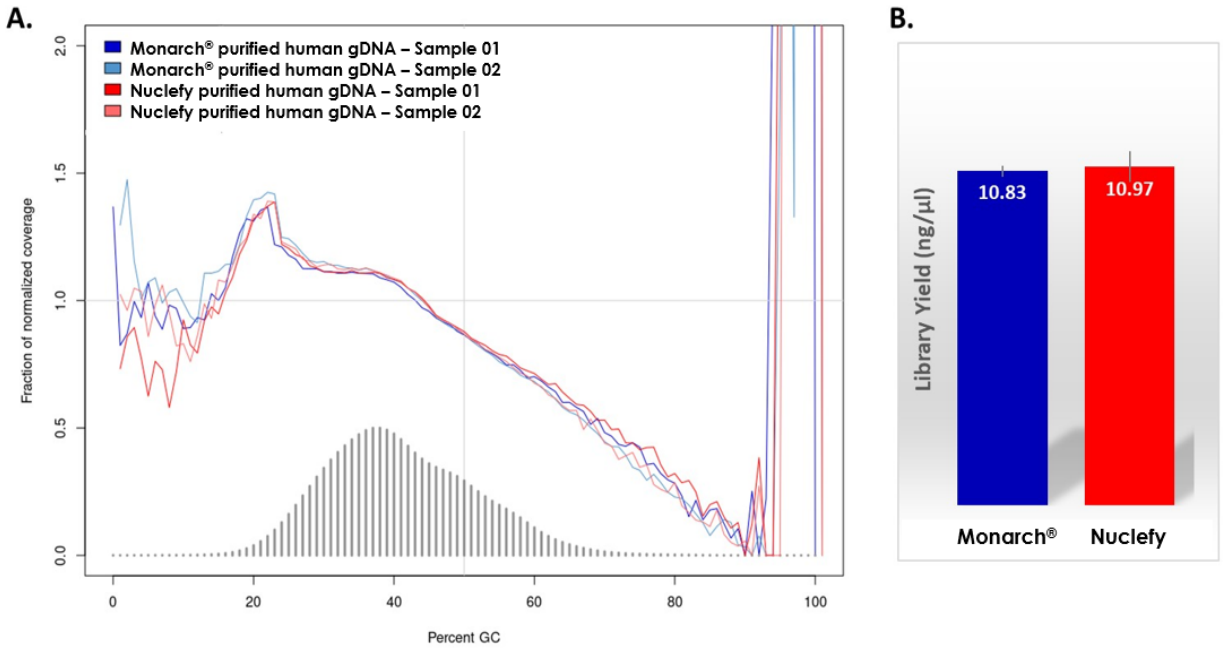

Figure 5. A GC bias plot. **B.** Library yield.

11. SAMPLE TYPE

The subsequent table shows detailed information of diverse sample types regarding recommended and maximal sample input as well as expected yield. Compliance with these specifications is strongly recommended, otherwise deviations in yield and purity may be expectable.

Sample Type	Recommended Sample Input	Expected Yield [µg] *	Maximal Sample Input
Tissue **			
Liver	10 mg	15 - 25	15 mg
Kidney	10 mg	15 - 20	10 mg
Spleen	10 mg	35 - 60	10 mg
Heart	10 mg	7 - 8	25 mg
Lung	10 mg	15 - 18	15 mg
Brain	10 mg	5 - 8	12 mg
Muscle	10 mg	4 - 6	25 mg
Blood ***			
Human (whole)	100 µl	1.5 - 4	100 µl
Cattle	100 µl	1.5 - 2.5	100 µl
Horse	100 µl	4 - 6	100 µl
Chicken (nucleated)	10 µl	30 - 35	10 µl
Cells			
HeLa	1 x 10^6 cells	6 - 8	5 x 10^6 cells
HEK293	1 x 10^6 cells	6 - 8	5 x 10^6 cells
NIH/3T3	1 x 10^6 cells	5 - 7	5 x 10^6 cells
Bacteria			
E. coli (Gram-negative)	2 x 10^9 cells	5 - 10	2 x 10^9 cells
R. terrae (Gram-positive)	2 x 10^9 cells	5 - 10	2 x 10^9 cells
Yeast			
Pichia pastoris	5 x 10^7 cells	0.6 - 0.7	5 x 10^7 cells
Saliva/Buccal Cells ****			
Saliva (human)	200 µl	3 - 4	500 µl
Buccal swab (human)	1 swab	4 - 6	1 swab

Table 3. Processable samples types, sample input and corresponding gDNA yield.

* Expected yield [µg] based on recommended sample input.

** gDNA data shown for frozen tissue samples. In addition, effective tissue extraction was performed with frozen tissue powder and PhoenixProtect (Procomcure Biotech)-stabilized tissue.

*** gDNA data shown for fresh blood samples stabilized with EDTA. Other anticoagulants (citrate, heparin), as well as comparison between fresh and frozen samples show comparable results.

**** Saliva and buccal swabs naturally lead to lower integrity due to partial presence of dead cell debris, and thus degraded gDNA.

12. SAMPLE STORAGE AND HANDLING GUIDELINES

Sample Storage

To ensure high quality of the isolated gDNA, fast processing of samples is highly recommended. If this is not possible, samples should ideally be stored according to the following recommendations.

Tissue Samples

- Stabilizing agents (e.g. PhoenixProtect, Procomcure Biotech) can be used for transportation (either at room temperature or on ice) and/or safe short-term storage at +4 °C or -20 °C. Stabilized tissue samples can also be used for aliquot preparation.
- Tissue samples can be disrupted using liquid nitrogen and stored as powder at -80 °C.
- Tissue samples can be stored without further processing (i.e. whole pieces) at -80 °C.

Blood Samples

- Fresh blood samples can remain stable for up to 7 days. DNA degradation occurs progressively in older samples resulting in loss of yield. Make sure that fresh blood samples are not older than a week and that they are stored properly.
- Frozen blood samples should be kept frozen, otherwise DNA degradation may occur. Proteinase K, RNase A and Lysis Buffer shall be added directly to the frozen blood samples. Start lysis immediately and allow samples to thaw during lysis incubation.

Cells, Bacteria and Yeast

- If direct processing is not possible/planned, storage of cell pellets at -20 °C, ideally -80 °C, is strongly recommended, independent of origin (cultured cells, bacteria, yeast).

Sample Handling

Tissue Samples

General Information

To ensure higher yield of isolated DNA, always use the recommended amount of starting material. In order to prevent partial DNA degradation, homogenization of tissue samples is of upmost importance. If homogenization is no option, tissue can be pulverized by liquid nitrogen or cut into the smallest possible pieces.

Cutting frozen tissue into small pieces is usually easier using a clean frozen tissue block before it is thawed. Cutting very small pieces when working with frozen samples is highly recommended, due to the presence of ice crystals, which can disrupt the cells and release endogenous nucleases.

After the addition of Lysis Buffer and Proteinase K, every reaction tube must be thoroughly mixed and sample lysis immediately initiated at +56 °C.

Stabilized Tissue

If the stabilized tissue samples were frozen, thaw them before processing. Remove the stabilizing agent from the external part of the tissue (e.g. with an absorbent paper). Perform homogenization of tissue (alternatively cut the stabilized tissue into small pieces). Weigh in the recommended sample input amount. Particular attention with stabilized samples is needed, as the presence of proteins with fibrous structure can persist after the completion of the lysis step. Remaining indigestible protein fibers can give the lysate a turbid appearance and block the silica membrane. To ensure better DNA yield and quality, it is advisable to centrifuge the lysate before loading on the column, even if it looks clear.

Frozen Tissue

Frozen Tissue Powder

Pre-cool reaction tubes to -80 °C or on dry ice. Transfer and weigh out the desired amount of frozen tissue powder. Keep the reaction tubes frozen (at -80 °C or on dry ice), to ensure that the tissue powder does not thaw.

Frozen Tissue

Transfer the appropriate amount of frozen tissue into a suitable tube. Perform homogenization on tissue. Alternatively, cut the tissue into small pieces. Weigh in the recommended sample input amount in a pre-cooled tube. Immediately process with the lysis step.

Fresh Tissue

Place a pre-cooled reaction tube on a micro scale. Cut fresh tissue into small pieces, transfer the pieces to the pre-cooled reaction tube and immediately proceed with the lysis step.

Cells, Bacteria and Yeast

Frozen Pellet

Thaw pellet slowly on ice. Loosen the pellet by flicking the tube several times before adding the appropriate buffer.

Fresh Pellet

No special treatment required. Process right away according to the manual instructions.

13. PRE-APPLICATION PREPARATION

Make sure to get familiar with the complete process regarding the extraction of the desired sample type before beginning the procedure.

GENERAL IMPORTANT NOTES BEFORE GETTING STARTED

- Dilute Nuclefy Wash Buffer concentrate with 100 % Ethanol (not supplied) as indicated on the bottle label and store at room temperature.
- RNase A treatment is only necessary if a low percentage of co-purified RNA will negatively affect downstream applications.
- Stabilized and fresh samples should be kept on ice to avoid potential degradation.
- When using an alternative to thermal mixer (e.g. heating block, incubator or water bath), vortex sample at a repetitive frequency of approx. 5-15 min (depending on the overall incubation time).
- All centrifugation steps should be carried out at room temperature (+15 to +25°C).

Several protocols require the availability of additional non-supplemented material and chemicals:

- PBS, phosphate buffered saline
- **Bacterial isolation**
  - 25 mg/ml Lysozyme diluted in ddH_2O or 10 mM Tris-HCl, pH 8.0
- **Insect isolation**
  - Microtube pestle
  - Tissue Lysis Buffer containing 1/10th volume EDTA
- **Yeast isolation**
  - Bead-mill homogenizer and bead-mill tube (mechanical disruption)
  - Suitable lytic enzyme and digestion buffer - recommendation: Zymolyase (enzymatic disruption)

14. EXTRACTION AND PURIFICATION PROTOCOLS

PART 1/2: SAMPLE LYSIS

Depending on the starting material, please follow the respective protocol:

Sample: Whole Blood (non-nucleated)

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.

1. **Transfer 100 µl whole blood to a 1.5 ml microcentrifuge tube.**
   If processing less than 100 µl whole blood, add pre-cooled PBS to bring the total volume to 100 µl. Avoid thawing when processing frozen aliquot samples.

2. **Add 10 µl Proteinase K, 3 µl RNase A and 100 µl Blood Lysis Buffer to the sample. Mix immediately by vigorous vortexing for 10 sec.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzymes and Blood Lysis Buffer.

3. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

4. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Nucleated Red Blood Cells

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.

1. **Transfer 10 µl whole blood to a 1.5 ml microcentrifuge tube.**
   If processing frozen aliquot samples, do not let them thaw and continue with the following step.

2. **Add 90 µl pre-cooled PBS. Mix by vortexing.**

3. **Add 10 µl Proteinase K and 3 µl RNase A. Mix by vortexing for 5 sec.**

4. **Add 100 µl Blood Lysis Buffer. Mix immediately by vigorous vortexing for 10 sec.**

5. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

6. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Tissue

- Set a thermal mixer or heating block to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.

Important: Frozen sample material must not thaw. Stabilized and fresh samples should be kept on ice to avoid potential degradation. Work on ice until the addition of Tissue Lysis Buffer and Proteinase K.

1. **Weigh in the recommended tissue amount (see Table 3, page 9) and transfer to a 1.5 ml microcentrifuge tube.**
   Prior tissue homogenization is strongly recommended. Alternatively, tissue must be cut into smallest possible pieces to allow an efficient lysis and to prevent DNA degradation.

2. **Add Proteinase K (see Table 4) and 200 µl Tissue Lysis Buffer. Mix by vigorous vortexing for 10 sec.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzyme and Tissue Lysis Buffer.

Tissue Type	Proteinase K
Brain, Kidney, Skin, Ear Clips	3 µl
All other tissues	10 µl

Table 4. Proteinase K volume required for efficient sample lysis.

3. **To reach complete tissue breakup, incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*): 5 min if processing homogenized tissue or tissue powder, 5 up to 30 min for tissue pieces.**
   To maximize yield and RNA removal, incubation time can be extended up to 3 h.

4. **When working with samples > 15 mg, centrifuge at maximum speed (> 12,000 x *g*) for 3 min to pellet debris. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.**

5. **Add 3 µl RNase A and mix by vortexing.**

6. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

7. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Insects

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Prepare the appropriate volume of modified Tissue Lysis Buffer (containing 1/10th volume of 0.5 M EDTA).

1. **Weigh in 10-20 mg insect material and transfer to a 1.5 ml microcentrifuge tube.**

2. **Use a microtube pestle to homogenize the insect material.**

3. **Add 10 µl Proteinase K and 200 µl Tissue Lysis Buffer (containing 1/10th volume of 0.5 M EDTA). Mix briefly by vortexing.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzyme and Tissue Lysis Buffer.

4. **To reach complete tissue breakup, incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 30-60 min.**
   To maximize yield and RNA removal, incubation time can be extended up to 3 h.

5. **Centrifuge for 3 min at > 12,000 x *g*.**

6. **Transfer the supernatant to a new 1.5 ml microcentrifuge tube without touching the pellet.**

7. **Add 3 µl RNase to the lysate (transferred supernatant) and mix by vigorous vortexing for 10 sec.**

8. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

9. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Gram-negative Bacteria

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.

Lysis can be performed with a time-saving enzymatic approach or following the regular lysis protocol:

Regular Lysis

1. **Harvest up to 2 x 10^9 Gram-negative bacteria and centrifuge the bacterial suspension at > 12,000 x g for 1 min. Discard the supernatant without touching the pellet.**
   Leave a minimal volume of supernatant in the tube to avoid complete drying of the pellet.

2. **Add 100 µl pre-cooled PBS. Resuspend the pellet brielfy by vortexing or pipetting up and down.**

3. **Add 10 µl Proteinase K and 200 µl Tissue Lysis Buffer. Mix by vigorous vortexing for 10 sec.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzyme and Tissue Lysis Buffer.

4. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 2 h.**
   To maximize lysis, incubation time can be extended up to 4 h.

5. **Add 3 µl RNase A and mix briefly by vortexing.**

6. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 5 min.**

7. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Enzymatic Lysis

- Prepare 25 mg/ml Lysozyme in ddH_2O or 10 mM Tris-HCl, pH 8.0.
- Set a second heating device (e.g. heating block) to +37 °C.

1. **Harvest up to 2 x 10^9 Gram-negative bacteria and centrifuge the bacterial suspension at > 12,000 x *g* for 1 min. Discard the supernatant without touching the pellet.**
   Leave a minimal volume of supernatant in the tube to avoid complete drying of the pellet.
2. **Add 90 µl pre-cooled PBS. Mix briefly by vortexing or pipetting up and down.**
3. **Add 10 µl Lysozyme solution (25 mg/ml) and mix briefly by vortexing.**
4. **Add 100 µl Tissue Lysis Buffer and mix by vigorous vortexing for 10 sec.**
5. **Incubate at +37 °C for 5 min.**
6. **Add 10 µl Proteinase K and mix briefly by vortexing.**
7. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**
8. **Add 3 µl RNase A and mix briefly by vortexing.**
9. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**
10. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Gram-positive Bacteria and Archaea

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.
- Prepare 25 mg/ml Lysozyme in ddH_2O or 10 mM Tris-HCl, pH 8.0.

1. **Harvest up to 2 x 10^9 Gram-negative bacteria and centrifuge the bacterial suspension at > 12,000 x *g* for 1 min. Discard the supernatant without touching the pellet.**
   Leave a minimal volume of supernatant in the tube to avoid complete drying of the pellet.
2. **Add 80 µl pre-cooled PBS. Resuspend the pellet briefly by vortexing or pipetting up and down.**
3. **Add 20 µl Lysozyme solution (25 mg/ml) and mix briefly by vortexing.**
4. **Add 100 µl Tissue Lysis Buffer and mix by vigorous vortexing for 10 sec.**
5. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 10 min.**
6. **Add 10 µl Proteinase K and mix briefly by vortexing.**
7. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**
8. **Add 3 µl of RNase A to the lysate and mix briefly by vortexing.**
9. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**
10. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Yeast

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.

Important: Lysis of yeast samples can be performed either by mechanical or enzymatic disruption.

Mechanical Lysis

- Pre-cool the appropriate volume of PBS.

1. **Harvest up to 5 x 10^7 yeast cells and centrifuge the suspension at > 12,000 x *g* for 1 min. Discard the supernatant without touching the pellet.**
   Leave minimal volume of supernatant in the tube to avoid complete drying of the pellet.
2. **Add 150 µl pre-cooled PBS. Resuspend the pellet briefly by vortexing or pipetting up and down.**
3. **Add 150 µl Tissue Lysis Buffer. Mix briefly by vortexing.**
4. **Transfer the whole sample to a bead-mill tube.**
5. **Perform the cell disruption according to the bead-mill manufacturer's instruction.**
6. **Transfer 200 µl of the homogenized cell lysate to a new 1.5 ml microcentrifuge tube.**
   Avoid the transfer of foam.
7. **Add 10 µl Proteinase K. Mix briefly by vortexing.**
8. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 30 min.**
9. **Add 3 µl RNase A. Mix briefly by vortexing.**
10. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 5 min.**
11. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Enzymatic Lysis

1. **Harvest up to 5 x 10^7 yeast cells and centrifuge the suspension at > 12,000 x *g* for 1 min. Discard the supernatant without touching the pellet.**
   Leave minimal volume of supernatant in the tube to avoid complete drying of the pellet.
2. **Add 100 µl the digestion buffer containing lytic enzyme (according to manufacturer's instructions; add 3 µl RNase A if not included). Resuspend the pellet briefly by vortexing or pipetting up and down.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzyme and digestion buffer.
3. **Incubate at +37 °C for 30 min.**
4. **Add 10 µl Proteinase K and mix briefly by vortexing.**
5. **Add 100 µl Tissue Lysis Buffer and mix by vigorous vortexing for 10 sec.**
6. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**
7. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Saliva

- Set a thermal mixer or an alternative heating device to +56 °C.
- Set a microcentrifuge to +4 °C (if possible).
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.

1. **Collect up to 500 µl saliva sample in a new 15 ml tube.**
   Work on ice until the addition of Lysis buffer.

2. **Add 1 ml pre-cooled PBS and mix briefly by vortexing.**

3. **Transfer the whole sample to a 1.5 ml microcentrifuge tube and centrifuge at 1,000 x *g* for 1 min.**

4. **Discard the supernatant without touching the pellet.**
   Leave a minimal volume of supernatant in the tube to avoid complete drying of the pellet.

5. **Add 1 ml pre-cooled PBS and mix briefly by vortexing.**

6. **Centrifuge at 1,000 x *g* for 1 min.**

7. **Discard the supernatant without touching the pellet.**

8. **Add 100 µl PBS, 10 µl Proteinase K and 3 µl RNase A. Mix briefly by vortexing.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzymes and PBS.

9. **Add 100 µl Cell Lysis Buffer and mix briefly by vortexing.**

10. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**

11. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Buccal Swab

- Set a thermal mixer or an alternative heating device to +56 °C.
- Set a microcentrifuge to +4 °C (if possible).
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.

1. **Transfer 1 ml pre-cooled PBS to a 1.5 ml microcentrifuge tube.**

2. **Transfer the buccal swab into a 1.5 ml microcentrifuge tube containing PBS. Break off the swabstick.**

3. **Mix by vigorous vortexing for 10 sec.**

4. **Remove the swab from the microcentrifuge tube. Centrifuge at 2,000 x *g* for 30 sec.**

5. **Discard the supernatant without touching the pellet.**

6. **Add 100 µl pre-cooled PBS, 10 µl Proteinase K and 3 µl RNase A. Mix briefly by vortexing.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzymes and PBS.

7. **Add 100 µl Cell Lysis Buffer and mix briefly by vortexing.**

8. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for a minimum of 30 min.**

9. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Sample: Cultured Cells

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.
- Pre-cool the appropriate volume of PBS.

1. **Harvest up to 5 x 10^6 cultured cells and centrifuge the suspension at > 1,000 x *g* for 1 min. Discard the supernatant without touching the pellet.**
   Leave a minimal volume of supernatant in the tube to avoid complete drying of the pellet.
   When working with a frozen cell pellet, thaw by flicking the tube several times before continuing.

2. **Add 100 µl pre-cooled PBS. Resuspend the pellet by carefully pipetting up and down.**

3. **Add 1 µl Proteinase K and 3 µl RNase A. Mix briefly by vortexing.**

4. **Add 100 µl Cell Lysis Buffer. Mix immediately by vigorous vortexing for 10 sec.**

5. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

6. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Genomic DNA Cleanup

- Set a thermal mixer or an alternative heating device to +56 °C.
- Pre-heat the appropriate volume of Elution Buffer to +60 °C.

Important: Depending on purification requirements, two gDNA cleanup protocols are provided.

Desalting/Buffer Exchange protocol: Removal of salts and buffer components (e.g. phenol extraction)
Enzymatic protocol: Removal of proteins and/or RNA

Desalting Buffer Exchange Cleanup

Note: If total DNA input is less than 100 ng, 10 µg/ml of carrier RNA (not supplied) may be added to the Binding Buffer for quantitative DNA retrieval.

1. **Add the DNA sample (maximum 200 µl) to a 1.5 ml microcentrifuge tube.**
   If processing less than 200 µl sample, add nuclease-free water to bring the total volume to 200 µl.

2. **Mix briefly by vortexing.**

3. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

Enzymatic Cleanup

1. **Add the DNA sample (maximum 200 µl) to a 1.5 ml microcentrifuge tube.**
   If processing less than 200 µl of sample, add Tissue Lysis Buffer to bring the total volume to 200 µl.

2. **Mix briefly by vortexing.**

3. **Add 1 µl Proteinase K and 1 µl RNase A. Mix briefly by vortexing.**

4. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

5. **Continue with Step 1 of Part 2/2: Binding and Elution (page 19).**

PART 2/2: BINDING AND ELUTION

1. **Add 400 µl Binding Buffer to the sample. Mix thoroughly by pulse-vortexing for 5-10 sec.**
   Thorough mixing is essential for optimal results.

2. **Centrifuge briefly to collect any drops from the inside of the lid.**

3. **Insert the Purification Column into a 2 ml Collection Tube.**

4. **Transfer the entire sample (~ 600 µl) to the column.**
   Avoid the transfer of foam and touching the upper column area (risk of salt contamination in the eluate).

5. **Centrifuge at 1,000 x *g* for 3 min to bind the gDNA. Without emptying the collection tube, centrifuge at maximum speed (> 12,000 x *g*) for another 1 min.**

6. **Discard the flow-through and the Collection tube.**

7. **Insert the Purification Column into a new 2 ml Collection Tube.**

8. **Add 500 µl Wash Buffer. Close the cap and invert several times.**
   Inverting the Spin Column prevents salt contamination in the eluate.

9. **Centrifuge at maximum speed (> 12,000 x *g*) for 1 min.**

10. **Discard the flow-through.**
    Tip: Tap collection tube on a paper towel to remove any residual buffer.

11. **Re-insert the Purification Column into the Collection Tube.**

12. **Repeat step 8-11 for a second wash step.**

13. **Centrifuge the Purification Column at maximum speed (> 12,000 x *g*) for 2 min.**
    It is important to dry the column before the elution. Residual ethanol reduces the elution efficiency and may interfere with downstream applications.

14. **Insert the Purification Column into a nuclease-free 1.5 ml microcentrifuge tube.**

15. **Add 100 µl Elution buffer (pre-heated to +60 °C) to the matrix and incubate for 1 min.**
    Avoid matrix disruption by any direct contact with the tip. Smaller elution volumes can be used (recommended range: 35-100 µl), allowing for more concentrated gDNA but a reduced yield.

16. **Centrifuge at maximum speed (> 12,000 x *g*) for 1 min.**

17. **Discard the Purification Column. Keep the microcentifuge tube containing the eluted gDNA.**

18. **Process eluted gDNA directly or store it at +4 °C to +8 °C. For long term storage, store at -80 °C.**

15. TROUBLESHOOTING GUIDE

Please use this guide to troubleshoot possible problems that may arise. For further assistance, please contact the technical support staff at support@procomcure.com.

TISSUE TYPE	POSSIBLE CAUSE	SOLUTION
CLOGGED COLUMN		
General	Incomplete breakup/ homogenization or lysis of the sample	Extend the time of sample breakup/homogenization and/or lysis.
		Centifuge the sample after homogenization and/or lysis step to pellet debris. Continue the isolation process with the supernatant.
	Sample overload	Reduce the amount of initial sample input material.
LOW DNA YIELD		
General	Inappropriate sample storage and handling	Store and process the sample according to sample handling guidelines.
	Insufficient elution	Increase the elution volume or repeat the elution step.
		Ensure that the Elution Buffer is pipetted to the center of column‘s membrane.
		Ensure that the Elution Buffer is pre-heated to +60 °C.
	Improper washing	Check for proper buffer preparation.
	Proteinase K amount	Verify the correct amount of Proteinase K for the respective tissue being processed.
	DNA degradation	Check troubleshooting guide section “DNA degradation“.
Tissue	Sample overload	Reduce the initial sample input (note: some tissues are very rich in gDNA content, e.g. liver, kidney, spleen).
	Tissue junks	Ensure a proper preparation of the sample input material (cut tissue into pieces or homogenize it) to minimize nuclease activity.
Blood	Old blood sample	Check sample handling guidelines.
	Formation of hemoglobin precipitate	Animal species with a high hemoglobin content may require reduction in Proteinase K and incubation time during lysis to prevent the formation of insoluble hemoglobin complexes.
Cells	Overgrown culture	Avoid overgrowing cultures (containing lyzed cells and degraded DNA).
	Cell lysis setup	Add Proteinase K and RNase A to the sample and mix well before adding the Cell Lysis Buffer (ensure proper mixing).
DNA DEGRADATION		
General	Inappropriate sample storage and handling	Store and process the sample according to the sample handling guidelines.
	Sample input	Reduce the initial sample input amount.
Tissue	Tissue junks	Ensure a proper preparation of the sample input material (cut tissue into pieces or homogenize it) to minimize nuclease activity.
Blood	Old blood sample	Check the sample handling guidelines.
Cells	Overgrown culture	Avoid overgrowing cultures (containing lyzed cells and degraded DNA).

TISSUE TYPE	POSSIBLE CAUSE	SOLUTION
SALT CONTAMINATION		
General	Carry-over of guanidine salt	Avoid the transfer of foam and touching the upper column area. Invert the spin column several times after the addition of Wash Buffer.
PROTEIN CONTAMINATION		
Tissue	Insufficient digestion	Extend the time of sample breakup/homogenization and/or lysis.
	Clogged membrane	Centifuge the sample after homogenization and/or lysis step to pellet debris. Continue the isolation process with the supernatant.
Blood	Formation of hemoglobin precipitate	Animal species with a high hemoglobin content may require reduction in Proteinase K and incubation time during lysis to prevent the formation of insoluble hemoglobin complexes.
	High hemoglobin content	Some blood samples are rich in hemoglobin (dark red color). A red color after the lysis step indicates the need to extend the lysis time by 3-5 min.
RNA CONTAMINATION		
Tissue	Sample overload	Reduce the amount of initial sample input material (especially DNA rich tissues: e.g. liver, kidney, spleen) to avoid the inhibition of the RNase A activity.
	Insufficient digestion	Extend the time of sample breakup/homogenization and/or lysis to increase the RNase A activity.
A260/A230 RATIO		
General	Variation of EDTA concentration in the eluate	Mostly negligible, high ratio (> 3.0 possible) can be consitent with highly pure samples. No negative effect on downstream applications.

Table 5. Troubleshooting guide.

16. RELATED PRODUCTS

	Content	Cat.No.
Nuclefy Genomic DNA Purification Kit	150 preps	TRAXSKU16073
Nuclefy Genomic DNA Purification Kit (Test)	10 preps	TRAXSKU16074
Aegis 96-well PCR Reaction Plate	10 plates	TRAXSKU30001
Aegis 384-well PCR Reaction Plate	10 plates	TRAXSKU30009
PhoenixDx® ABC qPCR Mastermixes	Test	TRAXSKU12011
PhoenixDx® qPCR Mastermix Alpha	500 rxn	TRAXSKU12012
PhoenixDx® qPCR Mastermix Bravo	500 rxn	TRAXSKU12013
PhoenixDx® qPCR Mastermix Charlie	500 rxn	TRAXSKU12014
PhoenixDx® RT-qPCR Mastermix Alpha	500 rxn	TRAXSKU12015
PhoenixDx® RT-qPCR Mastermix Bravo	500 rxn	TRAXSKU12016
PhoenixDx® RT-qPCR Mastermix Charlie	500 rxn	TRAXSKU12017

Table 6. Related Products.

Ordering Information

For ordering Nuclefy Genomic DNA Purification Kit, visit us at

www.traxlabsystems.com

or order via E-mail:

sales@traxconnects.com

trax LAB SYSTEMS

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