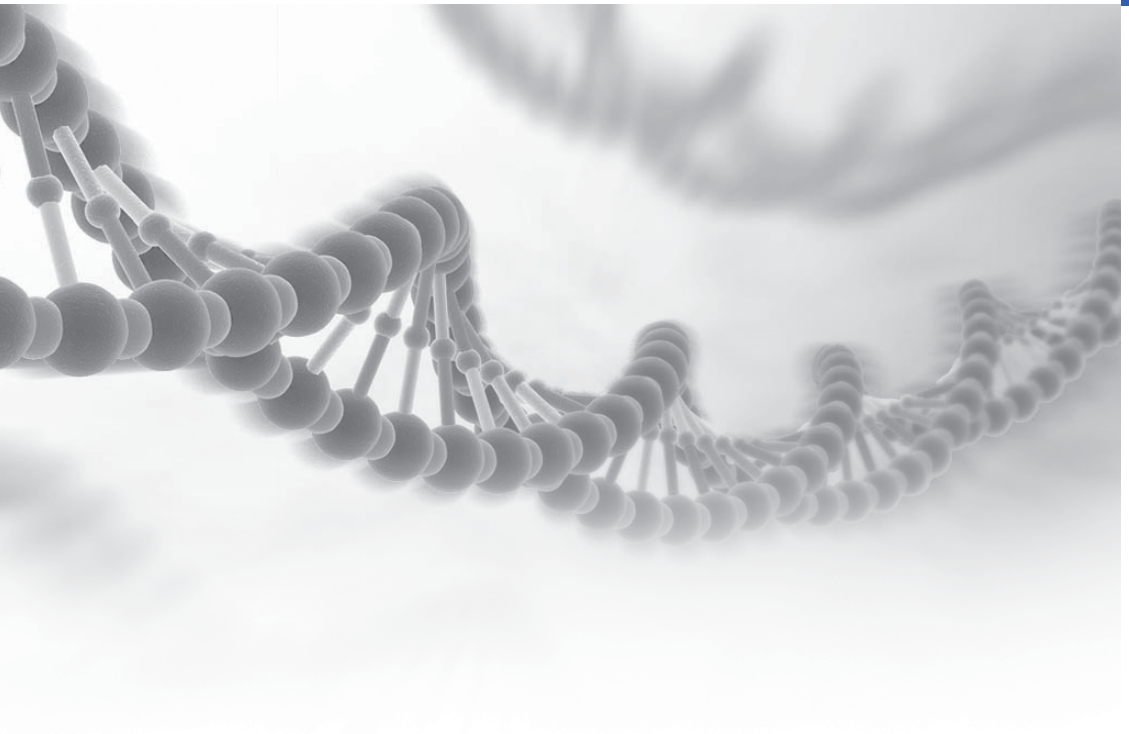
GENETIX BRAND

HANDBOOK

Nucleo-pore®



DNASure® Forensic Kit

☐ **DNASure® Forensic Kit**	NP-66505	50 Preps
☐ **DNASure® Forensic Kit**	NP-66507	250 Preps

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Nucleo-pore®

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COMPONENTS

Kit contents

DNASure® Forensic Kit

Cat. No.	NP-66505	NP-66507
Number of Preps	**50 preps**	**250 preps**
Lysis Buffer FBT1	20ml	100ml
Lysis Buffer FBT2	15 ml	75ml
Wash Buffer WBT	30 ml	2 X 75ml
Wash Buffer Concentrate WBT5	2 X7 ml	2 X 40ml
Elution buffer FEB	15ml	45ml
Proteinase K	30mg	2 X 75mg
Poteinase Buffer PB	1.8ml	8ml
Spin Columns	50	250
2ml Collection Tubes	100	500
Handbook	1	1

* Please see "Preparation of Reagents"

Reagents, consumables, and equipment not provided with the kit

- Absolute ethanol
- RNase A,
- 1.5 ml microcentrifuge tubes for sample lysis and DNA elution
- Disposable tips
- Pipettes
- Centrifuge 1.5-2ml rotor adaptor
- Vortex mixer
- Heating-block or dry bath for incubation at 70 °C
- Equipment for sample disruption and homogenization
- EZLyzer or Tissue Lyzer with stainless steel grinding jar sets
- Personal protection equipment (lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). Buffers FBT2 and WBT contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the DNASure® Forensic Kit:

Buffer FBT2
Contain Guanidine hydrochloride: R&S Phrases: R22-36/38

Buffer WBT
Contain Guanidine hydrochloride + isopropanol<25%: R&S Phrases: R10-22-36/38, S7-16-25

Proteinase K
Contain lyophilized Proteinase K: R&S Phrases: R22-36/37/38/42, S22-24-26-36/37
R10: Flammable, R22: Harmful if swallowed, R36/38: Irritating to eyes and skin, May cause sensitization by inhalation and skin contact, R36/37/38: Irritating to eyes, respiratory system and skin, R42: May cause sensitisation by inhalation, S7: Keep container tightly closed, S16: Keep away from sources of ignition - No smoking, S22: Do not breathe dust, S24: Avoid contact with skin, S25: Avoid contact with eyes, S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, S36/37: Wear suitable protective clothing and gloves

INTRODUCTION

Principle and Procedure

With the DNASure® Forensic Mini Kit method DNA can be prepared from tissues and Forensic samples. Lysis is achieved by incubation of the sample material in a proteinase K / SDS solution. Appropriate condition for binding of DNA to the silica membrane in the DNASure® Forensic Mini Kit Columns is achieved by the addition of chaotropic salts and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by subsequent washing with two different buffers. Pure DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Specifications of DNASure® Forensic Kit

DNASure® Forensic Mini Kit is designed for the fast, small-scale preparation of highly pure DNA from any forensic samples ,tissue, dried blood spots, buccal swab, nail scraping, cigarette butt, hair, bone, teeth and saliva. The purified DNA can be used directly for downstream applications like PCR, STR analysis, Southern blotting, or any kind of enzymatic reactions.

TheDNASure® Forensic Mini Kit Column is capable of binding up to 60 ug of genomic DNA.

Preparation and storage of reagents

Precautions:

Buffers FBT2 and WBT contain guanidine hydrochloride Always use personal protection equipments (PPE). All kit components can be stored at room temperature (18 – 25 °C) and are stable up to one year. During storage, especially at low temperatures, a white precipitate may form in Buffer FBT1, FBT2. Such precipitates can be easily dissolved by incubating the bottle at 50 – 70 °C before use.

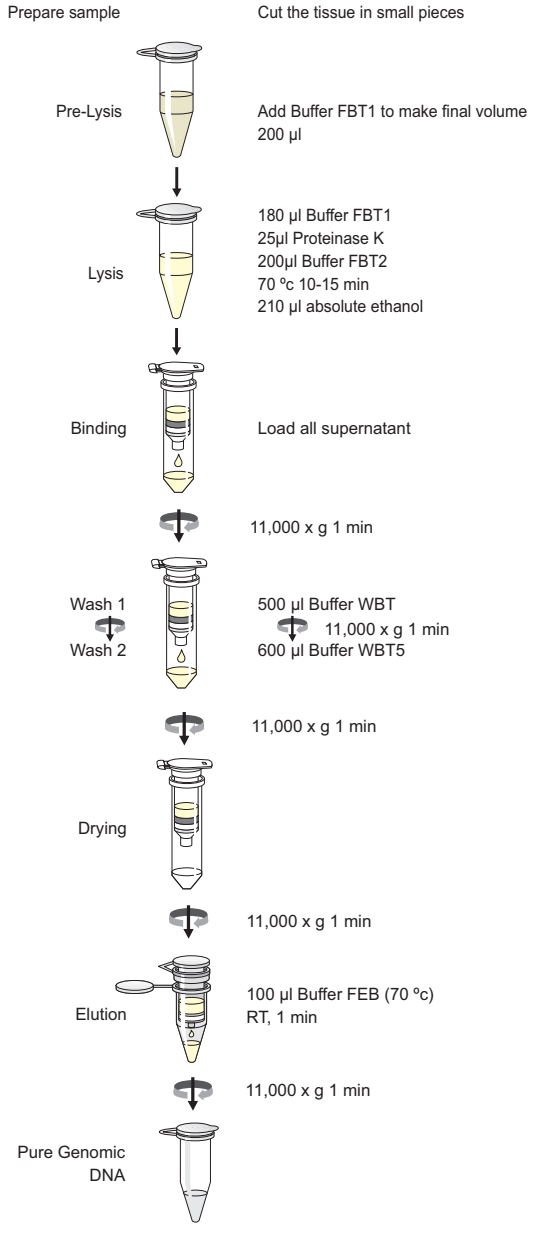
Wash Buffer WBT5

Reconstitute Wash Buffer WBT5 by adding 28ml and 160ml of ethanol (96-100%) to each bottle (Kits NP-66505 and NP-66507 respectively). Mark the bottle as "Ethanol Added". Store WBT5 at room temperature (18-25°C) for upto one year.

Proteinase K

Add 1.35ml of Proteinase Buffer PB to 30mg of lyophilized Proteinase K (Kit NP-66505).Similarly, add 3.35ml of Proteinase Buffer PB to 75mg of Proteinase K vial (Kit NP-66507). Reconstituted Proteinase K is stable at -20°C for upto 6 months.

Genomic DNA Purification



1. Protocol for DNA purification from tissue

DNA purification with DNASure® Forensic Mini Kit

Things to do Before starting:

- Check that Buffer WBT5 and Proteinase K were prepared as per instructions.
- Set dry bath or water bath to 56 °C.
- Preheat Elution Buffer FEB to 70 °C.

Protocol

1. Cut 25mg human or animal tissue into small pieces. Place the sample in a microcentrifuge tube (not provided) and proceed with step.

Note: Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer.

2. Add 180 µl Buffer FBT1 and 25 µl Proteinase K solution. Vortex. Make sure that the samples are completely covered with lysis solution. Incubate at 56°C until achieved for 1–3 hrs.

Vortex several times during incubation or use a shaking incubator.

If processing several samples, Proteinase K and Buffer FBT1 may be premixed directly before use. Do not mix Buffer FBT1 and Proteinase K more than 10 – 15 min before addition to the sample: Proteinase K tends to self-digestion in Buffer FBT1 without substrate.

Note: Incubation time can be extended to overnight if samples are not lyzed completely. If RNA-free DNA is crucial for downstream applications, an on column RNase digestion can be performed. Add 20ul RNase A (20 mg/ml) solution (not provided) and incubate for an additional 5 min at room temperature.

3. Vortex the samples. Add 200 µl Buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly. If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided).

4. Add 210 µl absolute ethanol to the sample and vortex vigorously.

After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.

5. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.

Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

6. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
7 Add 600 µl Buffer WBT5 to the Spin column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
8. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
9. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

2. Protocol for purification of DNA from dried blood spots (e.g. FTA® cards, Guthrie cards etc)

Starting material

Drying blood on filter paper is an effective form of storage and samples prepared in this manner are cheaper and safer to transport. A disc (3 mm diameter) punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.

Things to do before starting:

- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C.
- As filter paper tends to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample.

Protocol

1. For each dried blood sample, use the manual paper punch to cut out four 3 mm diameter discs. Transfer each set of 4 discs to a 2 ml sample tube.

2. Add 180 µl Buffer FBT1 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more Buffer FBT1 to the sample tube until the sample volume is 180 µl. Add 25 µl Proteinase K solution. Spin the samples briefly, vortex and incubate at 56°C for 1-2 hr. Pulse Vortex during incubation or use a shaking water bath. Make sure that the samples are completely covered with lysis buffer during incubation.
3 Add 200 µl Buffer FBT2, vortex vigorously to mix and incubate at 56°C for 10 min.

4. Add 210 µl absolute ethanol to the sample and vortex vigorously.
After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.

5. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.
Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

6. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
7. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
8. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
9. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

3. Protocol for purification of DNAfrom buccal swabs

Things to do before starting:

- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C.
- Make sure individual providing cheek cells has not taken food or drink at least 30 minutes prior to collection of sample.
- Collect the samples with cotton swab, or C.E.P. swabs (Gibco BRL). Scrape firmly against the inside of each cheek several times and let the swabs air dry.
- The respective individual should not have consumed food or drink within 30 min before collection of the sample.

Protocols

1. Place the dry swab material in 2 ml microcentrifuge tubes (not provided). Add 400 – 600 µl PBS and 25 µl Proteinase K solution to the swabs. The volume of PBS is depending on the type of swab used: for cotton and dacron, swabs, 400 µl are sufficient; for C.E.P. swabs, 600 µl are necessary. Mix by vortexing 2 x 5 s and incubate 10 min at 56 °C.
2. Transfer the lysate to a 1.5 ml fresh microcentrifuge tube.
3. Add one volume Buffer FBT2 (400 or 600 µl; depending on the swab type / volume of PBS buffer used) and vortex vigorously. Incubate the samples at 70°C for 10 min.

Note: Depending on the number of preparations, additional Buffer FBT2 might be needed .

4. Add one volume 96 – 100 % ethanol (400 or 600 µl, depending on the swab type) to each sample and mix by Pulse vortexing.
5. Transfer 600 µl of the samples from the 2 ml microcentrifuge tubes into Spin Columns. Centrifuge at 11,000 x g for 1 min. If the samples are not drawn through completely, repeat the centrifugation. Discard flow-through.

6 . Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
7. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
8. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
9. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

4. Protocol for DNA purification from Nail Scraping.

Starting material

The amount of biological sample material should not exceed 40 mg.

Things to do before starting:

- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C.

Protocol

1. Place the nail-scraping sample in a 2 ml sample tube. Add 180 µl Buffer FBT1 and 25 µl Proteinase K solution to the sample. Spin the samples briefly, vortex and incubate at 56°C for 30mins-1hr. Pulse Vortex during incubation or use a shaking water bath. Make sure that the samples are completely covered with lysis buffer during incubation.
2. Vortex the samples. Add 200 µl Buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly.
   If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided). If necessary, flick the tube to remove drops from inside the lid.
3. Add 210 µl absolute ethanol to the sample and vortex vigorously.
   After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.
4. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.
   Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
5. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
6. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
7. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
8. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.
   Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

5. Protocol for DNA purification from Cigarette Butt

Starting material

The amount of biological sample material (excluding the weight of the paper from the cigarette butt itself) should not exceed 40 mg.

Things to do before starting:

- As cigarette butts tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample
- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C.

Protocol

1. Place the cigarette-butt sample in a 2 ml sample tube. Add 180 µl diluted Buffer FBT1 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more Buffer FBT 1 to the sample tube until the sample volume is 180 µl.
   Add 25 µl Proteinase K solution. Spin the samples briefly, vortex and incubate at 56°C for 30mins – 1hr. Pulse Vortex during incubation or use a shaking water bath. Make sure that the samples are completely covered with lysis buffer during incubation.
2. Add 200 µl Buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly.
   If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided). If necessary, flick the tube to remove drops from inside the lid.
3. Add 210 µl absolute ethanol to the sample and vortex vigorously.

After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.

4. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.
   Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
5. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
6. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
7. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
8. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.
   Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

6. Protocol for DNA purification from Hair

Starting material

The amount of biological sample material should not exceed 25 mg. We recommend using 0.5–1 cm from the root ends of plucked hair samples.

Things to do before starting:

- Check that Buffer FBT2, Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C.

Protocol

1. Cut off the hair roots from the hair sample (up to 100) and collect them in a

1.5 mL microcentrifuge tube (not provided).

2. Place the hair sample in a 2 ml sample tube.Add 180 µL Buffer FBT1 to the hair roots , 25 µLProteinase K solution and 20 µl of 1 M DTT mix by vortexing, and incubate at 56 °C for 1-2hr . Use a shaking water bath or vortex occasionally.
3. Vortex the samples. Add 200 µl Buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly.

   If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided).
4. Add 210 µl absolute ethanol to the sample and vortex vigorously.

   After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.
5. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.

   Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
6. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
7. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
8. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
9. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperaturefor 1 min. Centrifuge 1 min at 11,000 x g.

   Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

7. Protocol for DNA purification from Bone & Teeth

Pretreatment for Bones or Teeth

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from bones or teeth. The protocol describes the preliminary grinding, decalcification using EDTA, and lysis of bone or teeth samples using proteinase K.

Starting material

The amount of biological sample material should not exceed 200 mg.

Things to do before starting:

- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C
- Refer to the EZLyzer or Tissue Lyzer Handbook.

Protocol

1. Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder using the EZLyzer or Tissue Lyzer system or an equivalent bead mill.

   When using the EZLyzer or Tissue Lyzer, transfer the bone sample and the ball into the grinding jar. Pre-chill the grinding jar by pouring liquid nitrogen over the ball and bone fragments. Allow the temperature to equilibrate . Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the EZLyzer or Tissue Lyzer (make sure you do not tighten the jars if liquid nitrogen is left in jars. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of bone).
2. Place 150–200 mg of powdered bone into a 2 ml microcentrifuge tube.
3. Decalcify by adding 600–700 µl 0.5 M EDTA (pH 8.3), and incubate at 37°C for 24–48 h.

   After incubation, set the temperature to 56°C for the next incubation step.
4. Centrifuge at 6000 rpm for 4 min. Transfer 200 µl of the supernatant to a new 2ml microcentrifuge tube.
5. Add 180 µl Buffer FBT 1 to the sample and 25ul of Proteinase K to the supernatant and incubate at 56°C for 3 h, or until the complete lysis of the supernatant.
6. Vortex the samples. Add 200 µl Buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly.

   If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided).

7. Add 210 µl absolute ethanol to the sample and vortex vigorously.
   After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.
8. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.
   Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
9. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
10. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
11. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
12. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.
    Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

8. Protocol for DNA purification from SALIVA

Starting material

The amount of biological sample material should not exceed 1ml

Things to do before starting:

- Check that Buffer WBT5, and Proteinase K were prepared as per instructions
- Set dry bath or water bath to 56°C.
- Before elution, preheat Elution Buffer FEB to 70°C

Protocol

1. Collect 1 ml saliva by spitting in a 50 ml sterile centrifuge tube. Or collect mouthwash in a 50 ml sterile centrifuge tube. Note: Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
2. Add 4 ml PBS (not provided) to the sample and centrifuge at 1800 x g for 5 min. Carefully decant the supernatant. Resuspend the pellet in 180 µl PBS. If RNA-free DNA is required, 20 µl of an RNase A stock solution (20 mg/ml) (not provided) should be added to the sample prior to the addition of Proteinase K and Buffer FBT1.
3. Add 25 µl Proteinase K and 180 µl BUFFER FBT1 to the sample. Mix immediately by vortexing for 15 s. In order to ensure efficient lysis, it is essential that the sample and buffer FBT1 are mixed immediately and thoroughly. Incubate at 56°C for 15-30 min.
4. Vortex the samples. Add 200 µl buffer FBT2, vortex vigorously and incubate at 70°C for 10 min. Vortex briefly.
   If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided).
5. Add 210 µl absolute ethanol to the sample and vortex vigorously.
   After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation. Make sure all of the precipitate is loaded onto the column.
6. For each sample, place one Spin Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000x g. Discard the flow-through and place the column back into the Collection Tube.
   Note: If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.
7. Add 500 µl Buffer WBT. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.
8. Add 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube.
9. Add another 600 µl Buffer WBT5 to the column and centrifuge for 1 min at 11,000 x g. Discardflow-through and place the column back into the Collection Tube. Centrifuge the column for 3 min at 11,000 x g. Residual ethanol is removed during this step.
10. Place the Spin Column into a 1.5 ml microcentrifuge tube (not provided) and add 50 µl prewarmed buffer FEB (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.
    Note: To increase yield, proceed with two elution steps with the same elution volume as indicated. Approximately 80% of the bound nucleic acid can be eluted. If high concentration is required, use 60% of the volume of elution buffer which increase the concentration of nucleic acid to 30%

TROUBLESHOOTING GUIDE

No or Poor DNA yield

Possible cause
- Incomplete lysis

Suggestion
- Sample not thoroughly homogenized and mixed with Buffer FBT1 /Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer FBT1. Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible cause
- Reagents not applied properly

Suggestions
- Prepare Buffer FBT2, Buffer WBT5, and Proteinase K solution as per instructions. Add ethanol to the lysates before loading them onto the columns.

Possible cause
- Suboptimal elution of DNA from the column

Suggestions
- Preheat Buffer FEB to 70 °C before elution. Apply Buffer FEB directly onto the center of the silica membrane.
- Elution efficiencies decrease dramatically, if elution is achieved with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer FEB (pH 8.5).
- Especially when expecting high yields from large amounts of material, we recommend elution with 50 µl Buffer FEB and incubation of the closed columns in dry bath at 70 °C for 5 min before centrifugation.

Poor DNA quality

Possible cause
- Incomplete lysis

Suggestions
- Sample not thoroughly homogenized and mixed with Buffer FBT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer FBT1.
- Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible cause
- Reagents not applied properly

Suggestions
- Prepare Buffer FBT2, Buffer WBT5, and Proteinase K solution according to instructions. Add ethanol to the lysates before loading them on the columns.

Possible cause
- RNA in sample

Suggestions
- If RNA-free DNA is desired, add 10 µl of RNase A solution (5 mg / ml; not supplied with the kit) before addition of Buffer FBT2 and incubate at 37°C for 5 min.

Clogged columns

Possible cause
- Too much sample material used

Suggestions
- Do not use more sample material than recommended (25 mg for most Protocol). If insoluble material like bones or hair remains in the lysate, spin down the debris and transfer the clear supernatant to a fresh microcentrifuge tube before proceeding with addition of Buffer FBT2 and ethanol.

Possible cause
- Incomplete lysis

Suggestions
- Sample not thoroughly homogenized and mixed with Buffer FBT1 / Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer FBT1.
- Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible cause
- Reagents not applied properly

Suggestions
- Prepare Buffer FBT2, Buffer WBT5, and Proteinase K solution according to instructions. Add ethanol to the lysates before loading them on the columns.

Suboptimal performance of genomic DNA in enzymatic reactions

Possible cause
- Carry-over of ethanol or salt

Suggestions
- Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer WBT5 before eluting the DNA. If, for any reason, the level of Buffer WBT5 has reached the column outlet after drying, repeat the centrifugation. Do not chill Buffer WBT5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer WBT5 to room temperature (18 - 25°C) before use.

Possible cause
- Contamination of DNA with inhibitory substances

Suggestions
- Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer FEB. If the A260 / A280 ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume Buffer FBT2 plus 1 volume ethanol (96 – 100 %) to the eluate. Load the mixture onto a Spin Column and proceed with step 5 of the standard protocol.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

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