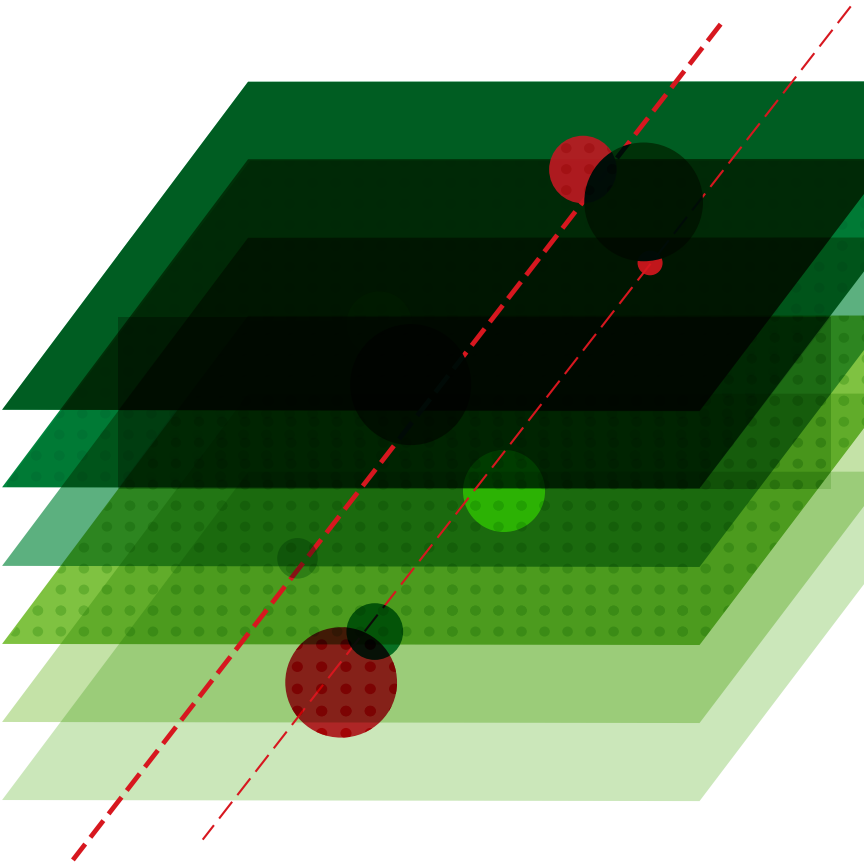
EXTRACTME®
GENOMIC DNA

Cat. No. EM13
Version: 1.2018

Kit for genomic DNA isolation from a variety of sample sources



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I. INTENDED USE

The ***EXTRACTME*** **GENOMIC DNA KIT** is designed for a rapid and efficient purification of high quality genomic, mitochondrial, bacterial, parasite or viral DNA from solid tissues (fresh, frozen, formalin-preserved or paraffin-embedded), physiological fluids (urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, sputum), fresh and frozen blood (human and mammalian), human and animal mucosa membrane swabs (including buccal, nasal, pharyngeal and vaginal swabs), semen, hair, rodent tails, insects, bacteria, yeast and cell cultures. The isolation protocol and buffer formulations have been optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	STORAGE CONDITIONS[1]
Catalogue number	EM13-010	EM13-050	EM13-250	
GL Buffer (Lysis Buffer)	3.8 ml	19 ml	94 ml	RT
⚠ **Proteinase K**** (lyophilized)	1 tube	1 tube	5 tubes	-20°C[2]
Proteinase Buffer	280 µl	1.4 ml	7 ml	RT
⚠ **RNase A*** (lyophilized)	1 tube	1 tube	5 tubes	-20°C[3]
RNase Buffer	100 µl	220 µl	1.1 ml	RT
GB Buffer (conc.)*** (Binding Buffer)	1.8 ml	10 ml	44 ml	RT
GW1 Buffer (conc.)*** (Wash Buffer 1)	3.3 ml	17 ml	82 ml	RT
GW2 Buffer (conc.)*** (Wash Buffer 2)	1.8 ml	9 ml	41 ml	RT
Elution Buffer	2 ml	10 ml	5 x 10 ml	RT
DNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5 x 50 pcs	RT

[1] RT – room temperature (+15°C to +25°C)

[2] After reconstitution, **Proteinase K** should be kept at -20°C.

[3] After reconstitution, **RNase A** should be kept at **+4°C for short term storage (several days) or in aliquots at -20°C**.

* Prior to the first use, add 220 µl RNase Buffer to a tube containing RNase A lyopilizate (in the kit for 10 isolations 100 µl of a buffer should be added).

** Prior to the first use, add 1.4 ml Proteinase Buffer to a tube containing Proteinase K lyopilizate (in the kit for 10 isolations 280 µl of a buffer should be added).

*** Prior to the first use, add appropriate amount of **96-100% ethanol** to **GB, GW1 and GW2 Buffer**; for details, see the instructions on the bottle label and in the table below. Marking the bottle after adding the alcohol is recommended.

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM13-010	EM13-050	EM13-250
GB Buffer	1.8 ml	10 ml	44 ml
96-100% ethanol	2.7 ml	15 ml	66 ml
Total volume	4.5 ml	25 ml	110 ml
GW1 Buffer	3.3 ml	17 ml	82 ml
96-100% ethanol	3.3 ml	17 ml	82 ml
Total volume	6.6 ml	34 ml	164 ml
GW2 Buffer	1.8 ml	9 ml	41 ml
96-100% ethanol	4.2 ml	21 ml	96 ml
Total volume	6 ml	30 ml	137 ml

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 96–100% PFA ethanol
- sterile microcentrifuge tubes (1.5–2 ml)
- automatic pipettes and sterile DNase-free tips
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- thermal heating block or water bath (up to 70°C)
- vortex mixer

Might be also needed:

- xylene – paraffin blocks
- PBS buffer – cell cultures, formalin preserved tissues, physiological fluids
  Preparation: dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml H_2O. Set the pH to 7.4 with HCl. Fill up to 1000 ml and autoclave. Store at +4°C.
- 1M DTT – hair & semen
  Preparation: dissolve 1.54 g DTT in 10 ml H_2O. Aliquot and store at -20°C.
- sterile swab sticks
- RBC Lysis Buffer (Red Blood Cell Lysis Buffer) (EM05-RBC) – whole blood
- YS Buffer (Spheroplast Buffer) (EM10-YS) and lyticase – yeast
- lysostaphin – *Staphylococcus* species
- lysozyme – *Enterococcus* species
- scissors, scalpel
- bead-beating tubes with ceramic filling (HPLM100, HPLM100a)
- tissue homogenizer for 2 ml tubes
- mechanical homogenizer with knives
- thermomixer (shaking orbit of 2 mm minimum)
- 50–75 ml smooth-stroke mortar with fitted piston
- liquid nitrogen or dry ice
- vortex mixer with a 2 ml tube adaptor
- centrifuge with a rotor for 10–15 ml tubes
  (physiological fluids, cell cultures)

IV. PRINCIPLE

DNA purification procedure consists of four steps and utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first step, biological material is lysed by Proteinase K in optimized GL Buffer. At this stage all the cellular membranes and proteins are degraded. When it is necessary to remove RNA the use of RNase A is recommended. After the addition of chaotropic salts, the lysate is applied to the purification minicolumn's membrane and the DNA is bound. The two-step washing stage effectively removes impurities and enzyme inhibitors. Purified DNA is eluted with the use of either a low ionic strength buffer (Elution Buffer) or water (pH 7.0–9.0) and may be used directly in all downstream applications such as PCR, quantitive real-time PCR, pharmacogenic research, Southern blotting, single-nucleotide polymorphism (SNP), short tandem repeat (STR) genotyping, DNA sequencing, enzymatic restriction, ligation and so forth, or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **GENOMIC DNA KIT** is tested with the use of standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

- fresh or frozen solid tissue: 1–30 mg
- formalin-preserved tissue: 1–30 mg
- paraffin-embedded tissue: 1–30 mg
- physiological fluids (urine, PMR, peritoneal fluid): up to 5 ml
- cell culture: 10^3–10^7 cells
- broth or plate bacterial or yeast culture, frozen cell pellet
- buccal, nasal, pharyngeal, vaginal, blood and saliva swabs or semen
- fresh or frozen blood: up to 1 ml
- hair: 10–30 mg
- insects: 1–30 mg

EFFICIENCY

The typical efficiencies of DNA isolation from fresh biological material are given in section XIII.

BINDING CAPACITY

50 µg DNA

TIME REQUIRED

- approx. 12 minutes (lysis time not included)
- 10–60 minutes for sample preparation

DNA PURITY

A_{260}/A_{280} ratio = 1.7–1.9

VII. SAFETY PRECAUTIONS

- Biological samples are treated as a biohazardous material of its potential pathogen content or health and life-threatening substances. While working with biological material it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- Use of sterile pipette filter tips is recommended.
- Avoid the cross-contamination of DNA between minicolumns.
- Guanidine salts' residues may form highly reactive components when combined with oxidation compounds. In case of spillage, clean the surface with a detergent-water solution.
- In case of spillage of a liquid containing microorganisms, clean the contaminated surface with a detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution

An optimal volume of Elution Buffer used should be chosen in accordance with the quantity of sample material and final DNA concentration expected.

Tissue: The use of 100–200 µl Elution Buffer is recommended when extracting from 2–10 mg of tissue or $<10^4$ cells. However, while extracting from 10–30 mg of tissue or 10^4–10^7 cells the volume of Elution Buffer should be increased to 200 µl.

Swab and Semen: The use of 50–100 µl Elution Buffer is recommended. The quantity of purified DNA depends on sample type and number of cells it contains; quality, however, on site's features the swab was taken from and interindividual diversity. Usually the isolation efficiency from a single buccal swab is equal to 1–3 µg of DNA whereas, from 150 µl of semen to 2–7 µg.

Bacteria and Yeast: The use of 50–100 µl Elution Buffer is recommended when extracting from no more than 10^9 cells (bacteria) or 10^8 cells (yeast). However, while extracting from a greater number of cells, the volume of elution buffer should be increased to 200 ul.

Blood: The use of 50–100 µl of Elution Buffer while extracting from 100–500 µl of blood is recommended. However, while extracting from 500–1000 µl of blood the volume of Elution Buffer should be increased to 200 ul.
The quantity of purified DNA depends on the type of sample and the number of white blood cells it contains (patient's age, his health condition, sample transport conditions, as well as storage time and method). Usually the isolation efficiency from 200 µl of blood sample from a healthy person is equal to 3–10 µg of DNA. A greater amount of DNA may be acquired from clinical samples containing increased number of white blood cells ($3x\ 10^6$–$1x\ 10^7$ cells/ml).

If a high DNA concentration is desired, the elution volume may be reduced. However, it may reduce the efficiency of the DNA retrieval. It is essential to apply Elution Buffer precisely to the centre of the membrane.

When isolating from a insignificant amount of sample material, use 200 µl Elution Buffer and precipitate the DNA according to standard procedures.

In order to maximize the DNA retrieval, heat Elution Buffer to 80˚C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, a second, 50–200 µl elution should be performed. For the second elution, repeat step 4 of the Isolation Protocol (see section XI), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions. If desired, DNA can alternatively be eluted from the minicolumn with nuclease-free water (pH 7.0–9.0) or TE Buffer.

RNA contamination

Most fresh or frozen tissue contains more RNA than DNA, especially metabolically active tissues like glands, nerve tissue and epithelium. RNA may interfere with some enzymatic reactions, but does not inhibit PCR. If an RNA-free DNA sample is desired, add 4 µl of RNase A solution and incubate at 37°C for 5 minutes (step 1 of the Isolation Protocol, section XI).

Foam formation in GL Buffer

The non-ionic detergent content of lysis buffer may cause a foam appearance after homogenization, vortexing or intensive pipetting. In order to eliminate the foam, centrifuge the buffer at 11 000 x g for 60 s.

IX. SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: 1–30 mg; **Sample material:** animal or human tissues (muscle, liver, heart, brain, kidney, bone marrow and others).

General procedure, applies to all homogenization methods

Divide a tissue into small fragments with tweezers and scissors or a scalpel. Follow one of the homogenization methods described below or go to step 1 of the Isolation Protocol (section XI).

Liquid nitrogen, dry ice (LN_2, CO_2)

1. Place tissue frozen in LN_2 or CO_2 in a previously chilled, sterile mortar. Using a chilled piston, carefully, but firmly crush tissue into smaller pieces and then, into a pulp.
2. Transfer the powder obtained this way into a 2 ml tube containing **375 µl GL Buffer** and go to step 1b of the Isolation Protocol (section XI).
   ⚠ *After pulping, a thin, sticky layer may be formed, rather than a powder. If this occurs, add 375 µl of GL Buffer to a mortar and reconstitute the tissue by pipetting and afterwards the lysate into a sterile, 2 ml tube. Remember to retrieve the tissue remains from the piston.*

Homogenization using a mechanical homogenizer equipped with knives

1. Place the tissue in a 2 ml tube, add **100 µl GL Buffer** and carefully homogenize with a sterile homogenizer tip.
2. After homogenization, retrieve the tissue remains from the knife tip by washing it with **275 µl GL Buffer**. Combine the fractions obtained this way and transfer the entire volume to a new 2 ml tube.
3. Continue the isolation from step 1b of the Isolation Protocol (section XI).

Homogenization using a bead-beating tube

We recommend the use of homogenization **bead-beating tubes** (HPLM100, HPLM100a) or ***EXTRACTME* DNA TISSUE PLUS KIT** (EM04), which contains tubes pre-filled with ceramic beads.

1. Add **150 µl GL Buffer** to a 2 ml ceramic bead-beating tube and suspend the sliced tissue in the buffer. Place the tube in a tissue homogenizer and homogenize at 3000–4000 x g for 30 s. If necessary, repeat the procedure.
   ⚠ *If evaluation of the degree of tissue fragmentation is compromised by foam formation, centrifuge the tube at 11 000 x g for 60 s.*
   ⚠ *If a tissue homogenizer is not available, the tissue may be homogenized by vortexing, using the appropriate 2 ml tube adaptor, for at least 5 minutes at maximum speed.*

2. Add **225 µl GL Buffer** and mix by pipetting.
3. Add **25 µl Proteinase K** and **4 µl RNase A**. Mix by vortexing for 20 s. Incubate at 37°C for 5 min.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

B. FORMALIN-PRESERVED TISSUE

Quantity: 1–30 mg; **Sample material:** animal tissues preserved in 4% formalin under cooling conditions.

1. Remove the formalin by washing it from the tissue two or three times, using PBS buffer or H_2O.
   ⚠ *Formalin is an irritating agent. Do not monitor its removal by inhaling the fumes from a tube.*
2. Continue the isolation following the procedure described for fresh or frozen solid tissue in section IXA.

C. PARAFFIN-EMBEDDED TISSUE

Quantity: 1-30 mg; **Sample material:** animal tissue embedded in paraffin block by standard histological procedure.

1. Prepare a fragment no larger than 30 mg by cutting it out of a paraffin block and place it in a 2 ml tube.
2. Add **1 ml xylene**. Mix by vortexing for 30 s.
   ⚠ *Xylene is toxic, irritating and very inflammable. Conduct the procedure in a working fume cupboard.*
3. Centrifuge at 15 000 x g for 5 min. Remove the supernatant by pipetting.
4. Repeat steps 2-3.
5. Add **1 ml 96–100% ethanol**. Mix by pipetting or vortexing for 15 s.
6. Centrifuge at 15 000 x g for 120 s. Remove the supernatant by pipetting.
7. Repeat steps 5–6.
8. In order to remove the remains of the ethanol, dry the pellet in the open tube at **50°C** for 5–20 min.
9. Add **375 µl GL Buffer** and mix by vortexing for 20 s.
10. Continue the isolation from step 1b of the Isolation Protocol (section XI).

D. CELL CULTURES

Quantity: 10^3–10^7 cells; **Sample material:** cell suspension or adherent cells, fresh or frozen at -80°C or -196°C.

1. Thaw frozen cells at **37°C**. Centrifuge the cells suspended in growth medium or PBS buffer in a 15 ml falcon tube or an 2 ml Eppendorf tube at 3 000 x g. If a compact cell pellet is not formed, wash the cells twice with **1 ml cold PBS buffer**.
2. Add **375 µl GL Buffer**. Mix thoroughly by vortexing for 30 s followed by pipetting.
   ⚠ *In some cases, where the cells tend to form either syncytia (myoblasts) or tight connections (epithelial cells) or they are high in number (approx. 10^7), it may be difficult to resuspend them in a GL Buffer. Should this happen, pipette carefully, using a ≥1000 µl pipette tip or a sterile syringe. Do not use filter tips.*
3. Transfer suspension obtained this way to a new 2 ml tube.
4. Continue the isolation from step 1b of the Isolation Protocol (section XI).

E. BACTERIA

Quantity: 0.2–3 ml (up to 10^9 cells); **Sample material:** broth and plate bacterial culture, frozen cell pellet.

Isolation from broth culture of Gram-negative bacteria (0.2-3 ml)

Before starting, always mix broth culture thoroughly. Transfer a desired quantity of bacterial culture (no more than 1.5 ml) to a sterile 1.5–2 ml Eppendorf tube and spin down the cells at 3000–4000 x g. Remove the supernatant. While isolating from a greater then 1.5 ml number of cultures, add an extra 1.5 ml of the culture to the top of the cell pellet obtained this way and spin it again. Resuspend the pellet in **375 µl of GL Buffer**. Continue the isolation following the Isolation Protocol from step 1b (section XI).

Isolation from more cells than recommended

When extracting DNA from a large number of cells (≥10^9), after spinning the culture, resuspend the cell pellet obtained this way in **450 µl of GL Buffer**, add **15 µl of Proteinase K** (step 1b), and subsequently **6 µl of RNase A** (step 1c). Continue the isolation following the Isolation Protocol from step 2 (section XI).

Isolation from frozen bacterial cells

Immediately after retrieving the frozen cell pellet from the freezer, resuspend it in **300 µl of GL Buffer**. Do not allow the cell pellet to thaw. Continue the isolation following the Isolation Protocol from step 1b (section XI).

Isolation from plate culture

Transfer **300 µl of GL Buffer** to a sterile 1.5 ml Eppendorf tube. Using a loop, take a sufficient amount of culture from the plate and resuspend it in GL Buffer. Continue the isolation following the Isolation Protocol from step 2 (section XI).

Isolation from Gram-positive bacteria

Gram-positive bacteria must be treated with an appropriate enzyme before commencing the isolation. For DNA isolation from *Staphylococci* use lysostaphin and from *Enterococci*, lysozyme.

Staphylococcus:

1. Pellet cells by centrifugation of 1.5 ml of broth culture*.
2. Discard the supernatant and suspend the cell pellet in **200 µl TE****. Mix thoroughly.
3. Add **30 µl lysostaphin 400 U/ml** solution and **4 µl RNase A**. Mix well by pipetting or vortexing.
4. Incubate at **37°C** for 20–60 min*** (until sample is lysed).
5. Add **300 µl GL Buffer** and **17 µl Proteinase K**. Mix thoroughly.
6. Incubate at **55°C** for 10 min.
7. Continue the isolation following the Isolation Protocol from step 2 (section XI).

* For thick cultures, use less broth cell culture.

** TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

*** When isolating from coagulase-negative strains, use 50 µl of lysostaphin and incubate 1h at 37°C.

Enterococcus:

1. Pellet cells by centrifugation of 1.5 ml of broth culture.
2. Discard the supernatant and suspend the cell pellet in **200 µl TE**, mix thoroughly.
3. Add **40 µl lysozyme 100 mg/ml** solution and **4 µl RNase A**, mix well by pipetting or vortexing.
4. Incubate at **37°C** for 40–60 minutes (until sample is lysed).
5. Add **300 µl GL Buffer** and **17 µl Proteinase K**, mix thoroughly.
6. Incubate at **55°C** for 10 min.
7. Continue the isolation following the Isolation Protocol from step 2 (section XI).

F. YEAST

Quantity: 0.2-3 ml (up to 10^8 cells); **Sample material:** broth and plate yeast culture, frozen cell pellet.

1. Pellet cells from 0.2–1.5 ml yeast broth culture by centrifugation for 5 min at 3 000-4 000 x g.
2. Discard the supernatant, and resuspend the cell pellet thoroughly in **200 µl YS Buffer** (EM10-YS Spheroplast Buffer – not included in the kit).
3. Add **50–200 U lyticase** and mix by vortexing.
4. Incubate at **30°C** for at least 30 min, mixing occasionally by inverting.
5. After incubation, centrifuge for 10 min at 1 000 x g.
6. Carefully, remove the supernatant with a pipette tip. DO NOT disturb the spheroplasts pellet!
7. Continue the isolation from step 1 of the Isolation Protocol (section XI).

G. PHYSIOLOGICAL FLUIDS

Quantity: up to 5 ml of fluid; **Sample material:** urine, cerebrospinal fluid, peritoneal fluid, pleural fluids, sputum.

⚠ Physiological fluids are a valuable diagnostic material, but also represent a great biological hazard due to the potential pathogen and/or cancer cell content. While working with physiological fluids it is essential to comply with all safety requirements for working with biohazardous material.

1. **Urine and other fluids:** depending on the volume, centrifuge in an appropriate tube at approx. 500 x g for 5 min. Discard the supernatant.
   Sputum: prior to centrifugation, add the appropriate quantity of a mucolytic agent (bromhexine, acetylcysteine). Centrifuge at 3000 x g for 5 min. Discard the supernatant.
2. Wash the cell pellet with **1 ml PBS buffer** or **saline**. Centrifuge at 3000 x g for 60 s.
3. Add **375 µl GL Buffer**. Mix thoroughly by vortexing for 30 s.
4. Continue the isolation from step 1b of the Isolation Protocol (section XI).

H. BLOOD

Quantity: up to 1 ml; **Sample material:** fresh or frozen blood.

1. Transfer 50–1000 µl of blood to a sterile 1.5–2 ml Eppendorf tube and add the same volume of **RBC Lysis Buffer** (EM05-RBC – not included in the kit).

For example, add 200 µl RBC Lysis Buffer to 200 µl blood. When isolating from less than 200 µl of blood, add Elution Buffer or PBS buffer in order to obtain a volume of 200 µl and then add 200 µl of RBC Lysis Buffer.

2. Mix well by inverting the tube until a clear red solution is obtained.
3. Centrifuge for 4 min at 8600 x g.
   ⚠ *Higher speeds are not recommended as they may hinder the subsequent suspension of the white blood cell pellet in lysis buffer.*
4. Carefully discard the supernatant from over the white blood cell pellet.
5. Resuspend the cell pellet completely in **375 µl GL Buffer** and vortex for 20 s.
6. Add **10 µl Proteinase K** and mix by inverting the tube several times or vortexing.
7. Incubate at **55°C** for 10 min.
8. Continue the isolation from step 2 of the Isolation Protocol (section XI).

I. SEMEN

Quantity: up to 150 µl; **Sample material:** semen.

1. Transfer 150 µl semen to a sterile, 1.5–2 ml Eppendorf tube.
   ⚠ *If the sample volume is less than 150 µl, add Elution Buffer or PBS buffer (not included in the kit) up to obtain a capacity of 150 µl. It should be noted that the efficiency of the DNA isolation will be lower.*
2. Add **375 µl GL Buffer, 10 µl Proteinase K** and **20 µl 1M DTT**, vortex for 3 s.
3. Incubate at **55°C** for 30 min. Mix by inverting the tube at several-minute intervals during the incubation.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

J. SWABS

Sample material: swabs (buccal, eye, nasal pharyngeal, vaginal and others).

1. Place the swab holding material in a 1.5 ml Eppendorf tube and cut off the excess from the end of the shaft so that the tube lid can be closed without difficulty.
2. Add **375 µl GL Buffer and 10 µl Proteinase K**, vortex for 3 s.
3. Incubate at **55°C** for 30 min. Mix by inverting the tube at several-minute intervals during the incubation.
4. Press the swab firmly against one side of the tube in order to retrieve the maximum possible volume of the lysate. Discard the swab.
5. Continue the isolation from step 2 of the Isolation Protocol (section XI).

K. HAIR

Quantity: 10–30 mg hair (100-120 strands), up to 30 mg of hair roots.
Sample material: hair, preferably with roots, or hair roots alone.

⚠ Hair roots contain living cells, while the rest of the hair bears only traces of degraded gDNA and mtDNA. Downstream applications such as PCR or qPCR should therefore involve small products ≤ 200 bp.

1. Cut off the hair roots and transfer them to a 2 ml tube. If the sample material does not contain hair roots, cut the hair into 3 mm fragments.
2. Add **375 µl GL Buffer**, **40 µl 1M DTT** and **25 µl Proteinase K**. Mix by vortexing for 30 s.
   ⚠ *The DTT supplement is optional. Most hair should be lysed without it; however, some hair types, such as curly, for instance, contain too many disulphide bridges for Proteinase K to handle.*
3. Incubate at **55°C** for at least 6 h, or overnight. Vortex for 60–120 s from time to time. A thermomixer may be used.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

L. RODENT TAILS

Quantity: up to 30 mg; **Sample material:** rat or mouse tail.

1. Cut the tail into smaller fragments and place in a 2 ml tube.
   ⚠ *For fragmentation instructions refer to the homogenization methods for fresh or frozen solid tissue (section IXA).*
2. Add **375 µl GL Buffer**. Mix thoroughly by vortexing for 20 s.
3. Add **4 µl RNase A** and **25 µl Proteinase K**. First incubate at **37°C** for 5 min and then at **55°C**, depending on the fragmentation' degree; 2–3 hours for well-homogenized samples or 5–16 hours for small fragments. Vortex vigorously for 20 s at least every 1–2 h. A thermomixer may be used.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

M. INSECTS

Quantity: 1–30 mg; **Sample material:** insects at various stages of life, fresh, frozen or preserved in formalin or ethanol.

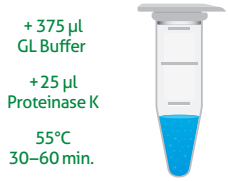
1. Wash insects preserved in formalin or ethanol twice with **PBS buffer** or **distilled water**. Centrifuge for 60 s at 500 x g. Depending on the fragmentation' degree go to step 1b or proceed with homogenization.
2. **Homogenization:** cut the insects into smaller fragments. Pound in a mortar with liquid nitrogen until a powder is obtained. Transfer the powder to a 2 ml tube. Homogenization may also be carried out using a tube with bead-beating filling (for instructions refer to section IXA).
   ⚠ *For fragmentation instructions refer to the homogenization methods for fresh or frozen solid tissue (section IXA).*
3. Add **375 µl GL Buffer** and vortex vigorously for 60 s.
4. Add **4 µl RNase A** and **25 µl Proteinase K**. Incubate at **37°C** for 5 min and then at **55°C**, depending on the fragmentation's degree; 2–3 h for well-homogenized samples, or 5-16 h for small fragments. Vortex vigorously for 20 s at least every 1-2 h. A thermomixer may be used.
5. Continue the isolation from step 2 of the Isolation Protocol (section XI).

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Prepare **Proteinase K** solution by reconstituting the lyophilizate in an appropriate quantity of Proteinase Buffer.
3. If necessary, reconstitute **RNase A** lyophilizate in an appropriate quantity of RNase Buffer.
4. Ensure that ethanol has been added to **GB**, **GW1** and **GW2 Buffers**. If not, add the appropriate quantity of **96–100% ethanol** (the volumes can be found on the bottle labels or in the table given in section II).
5. Examine the buffers. If a sediment has occurred in any of them, incubate it at **37°C** (**GB**, **GW1** and **GW2 Buffers**) or at **50–60°C** (other buffers) mixing occasionally until the sediment has dissolved. Cool to room temperature.
6. Set a dry block heater or water bath to 55°C.
7. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. ISOLATION PROTOCOL

STEP 1



Place the properly preparred biological material* in a 2 ml tube.

a. Add **375 µl GL Buffer** and vortex for 20 s.

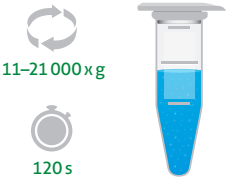
⚠ *See section IX. Sample preparation.

⚠ If a thick foam occurs, centrifuge the sample at 10 000 x g for 60-120 s. Refer to section VIII. Recommendations and Important Notes.

b. Add **25 µl Proteinase K** and mix by inverting the tube several times or vortexing. Incubate at **55°C** until the material has been completely digested. Mix-vortex vigorously for 20 s every 30-60 min.

c. If RNase A is to be used, add **4 µl RNase A** and incubate at **37°C** for 5 min.

STEP 2

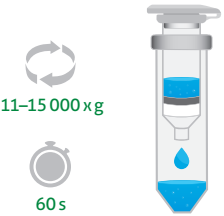


Add **400 µl GB Buffer** and mix thoroughly for 10 s.

Centrifuge for 120 s at **11 000–21 000 x g**.

Transfer the supernatant into a DNA Purification Column placed in a Collection Tube. Ensure no tissue remains are transferred along with it.

⚠ For homogenization with the use of bead-beating tubes: carefully pipet the appropriate volume of the supernatant by placing a 200 µl pipette tip (N.B.: a 1 ml tip may become clogged by the beads) into the filling. Tissue remains should either lie on one side of the tube or at the bottom.



Centrifuge for 60 s at **11 000–15 000 x g**.

⚠ The inside of DNA Purification Column should be dry after centrifugation. If any liquid remains in the upper part of the column, re-spin it for 120 s at maximum speed.

Transfer DNA Purification Column to a new 2 ml Collection Tube.

STEP 3

Add **600 µl GW1 Buffer** and centrifuge for 30 s at **11 000–15 000 x g**. Discard the filtrate and reuse Collection Tube.

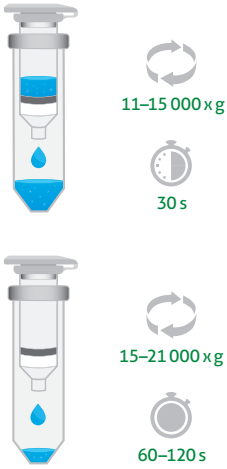
Add **500 µl GW2 Buffer** and centrifuge for 30 s at **11 000–15 000 x g**.

Discard the filtrate and reuse Collection Tube.

Centrifuge for 60–120 s at **15 000–21 000 x g**.

⚠ Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from DNA Purification Column before elution.

Discard Collection Tube and filtrate and carefully transfer DNA Purification Column to a sterile, 1.5 ml Eppendorf microcentrifuge tube.



STEP 4

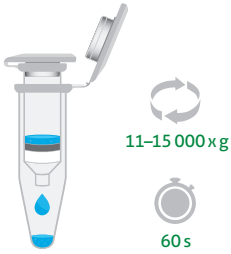
Add **50-200 µl Elution Buffer**, directly onto DNA Purification Column membrane.

⚠ Other buffer volumes in the 20–200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

Incubate DNA Purification Column at room temperature for 120 s.

Centrifuge at **11 000–15 000 x g** for 60 s.

Remove DNA Purification Column. Isolated DNA should be stored at +4°C or -20°C depending on further applications.



XII. TROUBLESHOOTING

Problem	Possible cause	Solution
TISSUE: Column becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that the Proteinase K solution is stored at -20°C. Extend the vortexing time, incubate the tissue with Proteinase K in GL Buffer for 16 h until the lysate is clear.
	Tissue remains were transferred onto the membrane.	Pipette the supernatant carefully, without disturbing the tissue pellet.
	DNA Purification Column is overloaded.	Do not exceed the recommended tissue amount or number of cell taken for DNA isolation.
SWAB: Column becomes clogged during purification.	The buccal swab sample contained food remains.	Repeat the isolation, ensuring that the person providing the sample does not consumed any food or drink for 30 minutes prior to the sample collection.
BACTERIA: Column becomes clogged during purification.	Incomplete cell lysis.	Reduce the amount of starting material or follow the instructions for the isolation from more than recommended cells. Extend the incubation time at 55°C to 20 min or until the cells are completely lysed.
	A thick slime of DNA was formed.	Repeat the centrifugation for 60 s at 15 000 x g or at the maximum speed.
BLOOD: Column becomes clogged during purification.	Improper storage of the sample material.	Storing blood samples at -80°C is highly recommended. Avoid subjecting the samples to repeated freeze/thaw cycles. Store blood samples at +4°C no longer than 24h.
	Incomplete white blood cell lysis.	Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 70°C, vortexing the lysate at several-minute intervals.
Purified DNA is degraded.	Old or damaged material was used.	Performing an isolation from fresh or properly preserved sample material is recommended.
	Inappropriate sample storage conditions.	Store samples at -80°C. Avoid subjecting the sample material to repeated freeze/thaw cycles.
	TISSUE: The DNA degraded as a result of over-intensive homogenization.	The recommended homogenization conditions should be applied (see section IXA).

Low yield of purified DNA.	The purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual GW2 Buffer is left in DNA Purification Column after centrifugation in step 3.
	DNA purification minicolumn has become clogged.	See "Column becomes clogged during purification".
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer directly to the centre of the membrane, heat it to 80°C. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase the volume of Elution Buffer to 200 µl.
	The pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
TISSUE: Low yield of purified DNA.	The tissue was incorrectly stored or preserved; DNA degradation.	Store tissue at -80°C. Avoid subjecting the sample material to repeated freeze/thaw cycles. Ensure that a good quality fixative is used and that the storage conditions are adequate.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in GL Buffer. The tissue must first be fragmented into the smallest possible pieces and homogenized by an appropriate method.
	Incomplete tissue lysis.	The tissue should be as well-fragmented as possible, increase the vortexing time, incubate the tissue with Proteinase K in GL Buffer for 16 h.
	Reduced Proteinase K activity.	Inappropriate storage conditions for Proteinase K. When applicable, ensure that the sample material is thoroughly washed with PBS buffer.
SWAB: Low yield of purified DNA.	Improper sample collection method. The buccal swab contains too few peeling cells.	When collecting a sample, ensure that the swab stick is scraped firmly against the inside of the cheek.
	Incomplete cell lysis.	Extend the incubation time at 55°C. Mix by inverting at several-minute intervals.
BACTERIA: Low yield of purified DNA.	Starting material contained few bacterial cells.	Increase the amount of starting material or decrease the volume of Elution Buffer to 20 µl.

BLOOD: Low yield of purified DNA.	Material contains few white blood cells.	Increase sample volume and/or decrease Elution Buffer volume to 20 µl.
	Incomplete white blood cell lysis.	Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 65°C, vortexing the lysate at several-minute intervals.
Isolated DNA is of low purity.	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
	The purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual GW2 Buffer is left in DNA Purification Column after final centrifugation step.
TISSUE: Isolated DNA is of low purity.	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that the solution is stored at -20°C. Extend the vortexing time, incubate the tissue with Proteinase K in GL Buffer for 16 h until the lysate is clear.
	Reduced Proteinase K activity.	Inappropriate storage conditions for Proteinase K. While working with the material described in the section IX (B, D, G, M), cell pellet sholud be thoroughly washed with PBS buffer to avoid inactivation of Proteinase K.
SWAB: Isolated DNA is of low purity.	The swab sample is of very low purity.	Use 600 µl of GW2 Buffer during second washing step (step 3 of Isolation Protocol), and centrifuge at 11 000-15 000 x g for 60 s. Empty Collection Tube and re-spin the dry minicolumn. In case of buccal swab ensure that the person providing the sample does not consume any food or drink during 30 minutes prior to the sample collection.
BLOOD: Some red blood cells are present in the white blood cell pellet.	The red blood cells have not been completely lysed.	Discard the supernatant from over the white blood cell pellet and repeat RBC lysis step. However, if a small quantity of red blood cells remain, this will not interfere with the subsequent DNA isolation steps.
RNA contamination present.	Sample material containing much RNA.	Perform digestion with RNase A (step 1 of the Isolation Protocol).
Inhibition of downstream enzymatic reactions.	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual GW2 Buffer is left in DNA Purification Column after centrifugation in step 3.

XIII. EXAMPLES OF AVERAGE ISOLATION EFFICIENCIES FROM FRESH BIOLOGICAL MATERIAL

SAMPLE MATERIAL	Mass /quantity	Elution volume	DNA conc.	A_{260}/A_{280}	Yield
Rat liver	30 mg	200 µl	235.2 ng/µl	1.86	47 µg
Rat skeletal muscle	20 mg	200 µl	50.5 ng/µl	1.80	10.1 µg
Rat heart	20 mg	200 µl	123.5 ng/µl	1.84	24.7 µg
Yellow adipose tissue	30 mg	200 µl	49 ng/µl	1.80	9.8 µg
Brown adipose tissue	30 mg	200 µl	116.7 ng/µl	1.88	23.3 µg
Rat kidney	20 mg	200 µl	183.4 ng/µl	1.79	36.7 µg
HT29 cell culture	1x 10^5	200 µl	51.5 ng/µl	1.81	10.3 µg
HCT116 cell culture	3x 10^6	200 µl	60 ng/µl	1.71	12.0 µg
Rat brain	20 mg	200 µl	9.4 ng/µl	1.69	1.88 µg
Insects	2.7 mg	30 µl	12.9 ng/µl	1.65	0.39 µg

XIV. EXAMPLES OF AVERAGE FRESH BIOLOGICAL MATERIAL PROCESSING TIMES

SAMPLE MATERIAL	Digestion in a block heater, no homogenization, periodical mixing	Digestion in a thermomixer	Liquid nitrogen homogenization	Homogenization with Bead-Beating Tubes	Approximate lysis time
Rat liver	3-6 h	2-4 h	1-2 h	≤ 1h	min 1 h, max 6 h
Rat skeletal muscle	2-3 h	2 h	≤ 1h	0.5-1 h	min 0.5 h, max 3 h
Rat heart	2-3 h	1-1.5 h	≤ 1h	0.5-1 h	min 0.5 h, max 3 h
Yellow adipose tissue	1-1.5 h	1 h	0.5 h	0.5 h	min 0.5h, max 1.5h
Brown adipose tissue	1-1.5 h	1 h	0.5 h	0.5 h	min 0.5h, max 1.5h
Rat kidney	1.5-3 h	0.5-2 h	0.5-1.5 h	0.5-1.5 h	min 0.5 h, max 3 h
HT29 cell culture	0.5-1 h	5-30 min	No data	app. 5 min	min 5 min, max 1 h
HCT116 cell culture	0.5-1 h	5-30 min	No data	app. 5 min	min 5 min, max 1 h
Rat brain	0.5-1 h	0.5-1 h	No data	No data	min 0.5 h, max 1 h
Insects	No data	1-2 h	0.5-2 h	0.5-2 h	min 0.5 h, max 2 h

XV. SAFETY INFORMATION

Proteinase K (lyophilized)

Danger
H315, H319, H334, H335
P261, P271, P304+P340, P342+P311
EUH208

GL Buffer

Warning
H319
P264, P305+P351+P338

GB Buffer

Danger
H302, H315, H318, H332, H412
P261, P280, P305+P351+P338, P301+P312 P330, P304+P340 P312

GW1 Buffer

Danger
H315, H318, H412
P280, P305+P351+P338, P310

EUH208 Contains Proteinase. May produce an allergic reaction. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H332** Harmful if inhaled. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **H412** Harmful to aquatic life with long-lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P271** Use only outdoors or in a well-ventilated area. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P342+P311** If experiencing respiratory symptoms: call a POISON CENTER or doctor/physician. **P304+P340** IF INHALED: Remove person to fresh air and keep comfortable for breathing. **P312** Call a POISON CENTER/doctor if you feel unwell. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. **P310** Immediately call a POISON CENTER/doctor.