Kit for DNA isolation from animal tissue and cell culture
I. INTENDED USE

The **EXTRACTME DNA TISSUE** kit is designed for the rapid and efficient purification of high quality DNA from solid tissues (fresh, frozen, formalin-preserved or paraffin-embedded), physiological fluids, hair, rodent tails, insects 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

<table>
<thead>
<tr>
<th>NUMBER OF ISOLATIONS</th>
<th>50 ISOLATIONS</th>
<th>150 ISOLATIONS</th>
<th>250 ISOLATIONS</th>
<th>3 ISOLATIONS (DEMO)</th>
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<tbody>
<tr>
<td>Catalogue number</td>
<td>EM03-050</td>
<td>EM03-150</td>
<td>EM03-250</td>
<td>EM03-D</td>
</tr>
<tr>
<td>Catalogue number*</td>
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<td>EM04-150*</td>
<td>EM04-250*</td>
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<tr>
<td>TL Buffer (Tissue Lysis Buffer)</td>
<td>20 ml</td>
<td>60 ml</td>
<td>100 ml</td>
<td>1.2 ml</td>
</tr>
<tr>
<td>Proteinase K (lyophilized)</td>
<td>1 pcs</td>
<td>3 pcs</td>
<td>5 pcs</td>
<td>1 pcs</td>
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<tr>
<td>Proteinase Buffer</td>
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<tr>
<td>RNase (lyophilized)</td>
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<td>5 pcs</td>
<td>1 pcs</td>
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<td>RNase Buffer</td>
<td>200 μl</td>
<td>600 μl</td>
<td>1 ml</td>
<td>100 μl</td>
</tr>
<tr>
<td>TB Buffer (conc.)**</td>
<td>8.8 ml</td>
<td>26.4 ml</td>
<td>44 ml</td>
<td>1.2 ml***</td>
</tr>
<tr>
<td>(Binding Buffer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TW1 Buffer (conc.)**</td>
<td>20 ml</td>
<td>60 ml</td>
<td>100 ml</td>
<td>1.2 ml***</td>
</tr>
<tr>
<td>(Wash Buffer 1)</td>
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<tr>
<td>TW2 Buffer (conc.)**</td>
<td>8.2 ml</td>
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<td>41 ml</td>
<td>1.5 ml***</td>
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<tr>
<td>(Wash Buffer 2)</td>
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<tr>
<td>Elution Buffer</td>
<td>10 ml</td>
<td>3 x 10 ml</td>
<td>5 x 10 ml</td>
<td>600 μl</td>
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<tr>
<td>DNA Purification Columns</td>
<td>50 pcs</td>
<td>3 x 50 pcs</td>
<td>5 x 50 pcs</td>
<td>3 pcs</td>
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<tr>
<td>Collection Tubes (2 ml)</td>
<td>50 pcs</td>
<td>3 x 50 pcs</td>
<td>5 x 50 pcs</td>
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<tr>
<td>Bead-Beating Tubes*</td>
<td>50 pcs</td>
<td>3 x 50 pcs</td>
<td>5 x 50 pcs</td>
<td>3 pcs</td>
</tr>
</tbody>
</table>

* Refers only to the **EXTRACTME DNA TISSUE PLUS** kit. The Bead-Beating Tubes have ceramic filling.

** Before using for the first time, add the appropriate quantity of 96-100% ethanol to the TB, TW1 and TW2 Buffers; for details, see the instructions on the bottle label and in the table below. Marking the bottle after adding the alcohol is recommended.

*** N.B.: the TB, TW1 and TW2 Buffers in the DEMO kit (cat. no. EM03-D and EM04-D) already contain ethanol.
Proteinase K and RNase A are shipped lyophilized. Each vial contains sufficient amount of enzyme for 50 DNA isolations. The RNase A and Proteinase K lyophilizates should be stored at +4°C.

Before using for the first time, reconstitute the RNase A lyophilizate in 200 μl of RNase Buffer (for the DEMO kit, reconstitute in 100μl of RNase Buffer). After reconstitution, the RNase A should be kept at +4°C for short-term storage (several days) or in aliquots at -20°C.

Reconstitute the Proteinase K lyophilizate in 1.3 ml of Proteinase Buffer (for the DEMO kit, reconstitute in 200 μl of Proteinase Buffer). Proteinase K solution should be stored at -20°C.

All the other components of the kit should be stored at room temperature (15-20°C). In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing. Under proper storage conditions, the kit will remain stable for at least 12 months.

<table>
<thead>
<tr>
<th>NUMBER OF ISOLATIONS</th>
<th>50 ISOLATIONS</th>
<th>150 ISOLATIONS</th>
<th>250 ISOLATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalogue number</td>
<td>EM03-050</td>
<td>EM03-150</td>
<td>EM03-250</td>
</tr>
<tr>
<td>Catalogue numer*</td>
<td>EM04-050*</td>
<td>EM04-150*</td>
<td>EM04-250*</td>
</tr>
<tr>
<td>TB Buffer</td>
<td>8.8 ml</td>
<td>26.4 ml</td>
<td>44 ml</td>
</tr>
<tr>
<td>96-100% ethanol</td>
<td>13.2 ml</td>
<td>39.6 ml</td>
<td>66 ml</td>
</tr>
<tr>
<td>Total volume</td>
<td>22 ml</td>
<td>66 ml</td>
<td>110 ml</td>
</tr>
<tr>
<td>TW1 Buffer</td>
<td>20 ml</td>
<td>60 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>96-100% ethanol</td>
<td>20 ml</td>
<td>60 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>Total volume</td>
<td>40 ml</td>
<td>120 ml</td>
<td>200 ml</td>
</tr>
<tr>
<td>TW2 Buffer</td>
<td>8.2 ml</td>
<td>24.6 ml</td>
<td>41 ml</td>
</tr>
<tr>
<td>96-100% ethanol</td>
<td>19.2 ml</td>
<td>57.6 ml</td>
<td>96 ml</td>
</tr>
<tr>
<td>Total volume</td>
<td>27.4 ml</td>
<td>82.2 ml</td>
<td>137 ml</td>
</tr>
</tbody>
</table>

* Refers only to the EXTRACTME DNA TISSUE PLUS kit.
III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

→ 96-100% ethanol PFA
→ 1.5-2 ml sterile microcentrifuge tubes
→ automatic pipettes and pipette tips
→ disposable gloves
→ microcentrifuge with rotor for 1.5-2 ml (≥11k x g)
→ dry block heater or water bath (up to 70°C)
→ vortex mixer

May be necessary:
→ xylene – paraffin blocks
→ PBS buffer – cell cultures, formalin preserved tissues, physiological fluids
  Preparation: dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄ and 0.24 g KH₂PO₄ in 800 ml H₂O. Set the pH to 7.4 with HCl. Fill up to 1000 ml and autoclave. Store at +4°C.
→ 1M DTT – hair
  Preparation: dissolve 1.54 g DTT in 10 ml H₂O. Aliquot and store at -20°C.
→ scissors, scalpel
→ bead-beating tubes with ceramic filling (cat. no. HPLM100)
→ 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

The DNA purification procedure consists of four steps and utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first isolation step, the tissue is homogenized in order to disintegrate intercellular bonds (epithelial tissue) and fragmentize high-molecular proteins (muscle or connective tissue). Then the homogenate is lysed by Proteinase K in optimized TL Buffer. At this stage, all the cellular membranes and proteins are degraded. When a metabolically active tissue is used for isolation, RNA is removed by the RNase A. The homogenate is separated from the undigested tissue remains by centrifugation and combined with chaotropic salts. The mixture is then applied to the purification minicolumn membrane and the DNA is bound. The two-step washing stage effectively removes impurities and enzyme inhibitors. The purified DNA is eluted using a low ionic strength buffer or water (pH of 7.0-9.0) and can be used directly in all downstream applications such as qPCR, Southern blotting, DNA sequencing, enzymatic restriction, DNA ligation and so forth, or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the EXTRACTME DNA TISSUE kit is tested using standard QC procedures. The purified DNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer. In addition, the functional quality is tested by qPCR and digestion with restriction enzymes.
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
- cell culture: $10^3$-$10^7$ cells
- physiological fluids (urine, PMR, peritoneal fluid): 1-5 ml
- hair: 1-30 mg
- insects: 1-30 mg

EFFICIENCY

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

BINDING CAPACITY

Approx. 50 μg DNA

TIME REQUIRED

- approx. 12 minutes (lysis time not included)
- 30-40 minutes for mechanical homogenization
- 1-16 hours for periodical shaking by vortexing

DNA PURITY

$A_{260}/A_{280}$ ratio = 1.7 – 1.9

VII. SAFETY PRECAUTIONS

- Tissue is treated as a biohazardous material on account of its potential pathogen content or health and life-threatening substances. While working with tissue and cell cultures, compliance with all the safety requirements for working with biohazardous material is essential.

- Conducting the entire isolation procedure in a Class II Biological Safety Cabinet or at a laboratory burner is recommended, as is wearing disposable gloves and a suitable lab coat.

- The use of sterile pipette filter tips is recommended.

- Avoid cross-transferral of DNA between minicolumns.

- Guanidine salts residues may form highly reactive compounds when combined with oxidation compounds. In case of spillage, clean the surface with a detergent-water solution.
VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution
The optimal volume of the elution buffer used should be chosen in line with the amount of the sample material and the final DNA concentration expected. The use of 100-200 μl Elution Buffer is recommended when extracting from 2-10 mg of tissue or <10^4 cells, as is increasing the elution buffer volume to 200 μl when isolating from 10-30 mg of tissue or 10^4-10^7 cells.

If a high DNA concentration is desired, the elution volume may be reduced to 50 μl. It should be noted that this may reduce the efficiency of the DNA retrieval. It is essential to apply the elution buffer precisely to the centre of the membrane.

When isolating from a small 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 the Elution Buffer to 80°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, a second, 200 μl elution should be performed. For the second elution, repeat steps 15 - 18 of the Isolation Protocol (see section XI), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

The Elution Buffer contains 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 5-10 mM of Tris Buffer, pH 8.0-9.0.

RNA contamination
Most fresh or frozen tissue contains more RNA than DNA, particularly in the case of metabolically active tissues, which are 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 3 of the Isolation Protocol, section XI).

Foam formation in the TL Buffer
The non-ionic detergent content of the lysis buffer may cause a foam appear after homogenization, vortexing or intensive pipetting. In order to eliminate the foam, centrifuge at 11k x g for 1 minute.
IX. SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: 1-30 mg; Sample material: animal or human tissues.

General procedure, applies to all homogenization methods
Divide the 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 the tissue into smaller pieces and then, into a pulp.
2. Transfer the powder thus obtained into a 2 ml tube containing 375 μl TL Buffer and go to step 2 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 the TL Buffer to a mortar and reconstitute the tissue by pipetting and then transfer the lysate into a sterile, 2 ml tube. Remember to retrieve the tissue remains from the piston as well.

Homogenization using a mechanical homogenizer equipped with knives

1. Place the tissue in a 2 ml tube, add 100 μl TL 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 TL Buffer. Combine the fractions thus obtained and transfer the entire volume to a new 2 ml tube.

Homogenization using a bead-beating tube

We recommend EXTRACTME DNA TISSUE PLUS (cat. no. EM04), which contains tubes pre-filled with ceramic beads.

1. Add 150 μl TL 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 3-4k 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 11k x g.
   △ 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 255 μl TL 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.


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 H₂O.
   - Formalin is an irritating agent. Do not monitor the formalin removal by smelling the fumes from the 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. Cut out a fragment no larger than 30 mg from the 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 15k 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 15k x g for 2 min. 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 TL Buffer and mix by vortexing for 20 s.

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 3k x g. If a compact cell pellet is not formed, wash the cells twice with 1 ml cold PBS buffer.
2. Add 375 μl TL 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 TL Buffer. In such cases, pipette carefully, using a ≥1000 μl pipette tip or a sterile syringe. Do not use filter tips.
3. Transfer everything to a new 2 ml tube.

E. PHYSIOLOGICAL FLUIDS

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

For isolation from blood samples, the dedicated *EXTRACTME DNA BLOOD* kit (cat. no. EM05) is recommended. For isolation from swabs or semen, the *EXTRACTME DNA SWAB & SEMEN* kit (cat. no. EM06) is proposed.

- 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, compliance with all the safety requirements for working with biohazardous material is essential.

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 3k x g for 5 min. Discard the supernatant.
2. Wash the cell pellet with 1 ml PBS buffer or saline. Centrifuge at 3k x g for 1 min.
F. 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 has 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 TL 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 the Proteinase K to handle.
3. Incubate at 55°C for at least 6 h, or overnight. Vortex for 1-2 minutes from time to time. A thermomixer may be used.
4. After the lysis is complete, continue the isolation from step 5 of the Isolation Protocol (section XI).
   ▲ For small quantities of sample material, elution in 200 μl Elution Buffer, followed by precipitation, is recommended.

G. 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 TL Buffer. Mix thoroughly by vortexing for 20 s.
3. 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 degree of fragmentation; 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.
H. 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 the PBS buffer or distilled water. Centrifuge for 1 min at 500 x g. Depending on the degree of fragmentation go to step 3 or proceed with homogenization (step 2).
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 TL Buffer and vortex vigorously for 1 min.
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 degree of fragmentation; 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 5 of the Isolation Protocol (section XI).
   ▲ For small quantities of sample material, elution in 200 μl Elution Buffer, followed by precipitation, is recommended.

X. BEFORE STARTING

1. Mix well each buffer supplied with the kit.
2. Prepare the Proteinase K solution by reconstituting the lyophilizate in an appropriate quantity of the Proteinase Buffer.
3. If necessary, reconstitute the RNase A lyophilizate in an appropriate quantity of the RNase Buffer.
4. Ensure that ethanol has been added to the TB, TW1 and TW2 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 (TB, TW1 and TW2 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. Heat a sufficient amount of the Elution Buffer to 70°C.
8. Unless otherwise stated, conduct all the isolation steps at room temperature.
XI. ISOLATION PROTOCOL

1. Place the fragmented biological material in a 2 ml tube. Add **375 μl TL Buffer** and vortex for 20 s.
   ▲ If a thick foam occurs, centrifuge the sample at 11k x g for 1-2 min. Refer to section VIII. Recommendations and Important Notes.

2. Add **25 μl Proteinase K** and mix by inverting the tube several times or vortexing.

3. Incubate at **55°C** until the material has been completely digested. Mix-vortex vigorously for 20 s every 30 - 60 min.

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

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

6. Centrifuge for 2 min at 11-21k x g.

7. Transfer the **supernatant** into a purification minicolumn placed in a collection tube. Ensure no tissue remains are transferred along with it.
   ▲ For homogenization using 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.

8. Centrifuge for 1 min at 11-15k x g.
   ▲ The inside of the purification column should be dry after centrifugation. If any liquid remains in the upper part of the column, re-spin it for 2 min at maximum speed.
9. Transfer the purification column to a new 2 ml collection tube.

10. Add **600 μl** TW1 Buffer and centrifuge for 30 s at 11-15k x g. Discard the filtrate and reuse the collection tube.

11. Add **500 μl** TW2 Buffer and centrifuge for 30 s at 11-15k x g.

12. Discard the flow-through and reuse the collection tube.

13. Centrifuge for 1-2 min at 15-21k x g.
   ▲ The 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 the minicolumn before elution.

14. Discard the collection tube and flow-through and carefully transfer the purification minicolumn to a sterile, 1.5 ml Eppendorf microcentrifuge tube.

15. Add **100-200 μl** Elution Buffer, pre-heated to 70°C, directly onto the purification minicolumn membrane.
   ▲ Other buffer volumes in the 20-200 μl range may be used. For instructions, see section VIII. Recommendations and important notes.

16. Incubate the minicolumn at room temperature for 2 min.

17. Centrifuge at 11-15k x g for 1 min.

18. Remove the minicolumn. The isolated DNA is ready for use in downstream applications or for either short-term storage at +4°C or long-term storage at -20°C.
### XII. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column becomes clogged during purification.</strong></td>
<td>Inappropriate tissue homogenization.</td>
<td>Select the appropriate homogenization conditions (see section IXA).</td>
</tr>
<tr>
<td></td>
<td>Incomplete protein degradation.</td>
<td>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 the Proteinase K in the TL Buffer for 16 h until the lysate is clear.</td>
</tr>
<tr>
<td></td>
<td>Tissue remains were transferred onto the membrane.</td>
<td>Pipette the supernatant carefully, without disturbing the tissue pellet.</td>
</tr>
<tr>
<td></td>
<td>The purification column is overloaded.</td>
<td>Do not exceed the recommended tissue amount or number of cell taken for DNA isolation.</td>
</tr>
<tr>
<td><strong>Low yield of purified DNA.</strong></td>
<td>The tissue was incorrectly stored or preserved; DNA degradation.</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Insufficient fragmentation of the sample material.</td>
<td>Ensure proper tissue homogenization in the TL Buffer. The tissue must first be fragmented into the smallest possible pieces and homogenized by an appropriate method.</td>
</tr>
<tr>
<td></td>
<td>Incomplete tissue lysis.</td>
<td>Ensure optimal conditions for Proteinase K activity. The tissue should be as well-fragmented as possible, increase the vortexing time, incubate the tissue with Proteinase K in the TL Buffer for 16 h.</td>
</tr>
<tr>
<td></td>
<td>Reduced Proteinase K activity.</td>
<td>Inappropriate storage conditions for the Proteinase K. When applicable, ensure that the sample material is thoroughly washed with the PBS buffer (see section IX B, C, E and H).</td>
</tr>
<tr>
<td></td>
<td>The purification minicolumn has become clogged.</td>
<td>See “Column becomes clogged during purification”.</td>
</tr>
<tr>
<td></td>
<td>Incomplete DNA elution from the membrane.</td>
<td>Before applying the Elution Buffer to the membrane, heat it to 80°C. Apply the Elution Buffer directly to the centre of the membrane. Extend the incubation time with the Elution Buffer to 10 min. Perform second elution. Increase volume of the Elution Buffer to 200 μl.</td>
</tr>
<tr>
<td></td>
<td>The pH of the water used for elution is lower than 7.0.</td>
<td>Use the Elution Buffer for DNA elution.</td>
</tr>
<tr>
<td><strong>Isolated DNA is of low purity.</strong></td>
<td>Incomplete protein degradation.</td>
<td>Prepare a fresh Proteinase K solution. Ensure that the solution is stored at -20°C. Extend the vortexing time, incubate the tissue with the Proteinase K in the TL Buffer for 16 h until the lysate is clear.</td>
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<tr>
<td>---------------------------------</td>
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<td>----------------------------------------------------------------------------------------------------------------------------------</td>
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<tr>
<td><strong>Reduced Proteinase K activity.</strong></td>
<td>Inappropriate storage conditions for the Proteinase K. When applicable, ensure that the sample material is thoroughly washed with the PBS buffer (see section IX B, C, E and H).</td>
<td></td>
</tr>
<tr>
<td><strong>Purified DNA is degraded.</strong></td>
<td>Old or damaged material was used.</td>
<td>Performing an isolation from fresh or properly preserved tissues is recommended.</td>
</tr>
<tr>
<td></td>
<td>Inappropriate tissue storage conditions or improper preservation.</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>The DNA degraded as a result of over-intensive homogenization.</td>
<td>The recommended homogenization conditions should be applied (see section IXA).</td>
</tr>
<tr>
<td><strong>RNA contamination present.</strong></td>
<td>Tissue containing much RNA.</td>
<td>Perform digestion with the RNase A (step 4 of the Isolation Protocol).</td>
</tr>
<tr>
<td><strong>Inhibition of downstream enzymatic reactions.</strong></td>
<td>Purified DNA contains residual alcohol.</td>
<td>Repeat the isolation, giving particular attention to ensuring that no residual TW2 Buffer is left in the purification column after centrifugation in step 13.</td>
</tr>
</tbody>
</table>
XIII. AVERAGE ISOLATION EFFICIENCIES FROM FRESH BIOLOGICAL MATERIAL

<table>
<thead>
<tr>
<th>SAMPLE MATERIAL</th>
<th>Mass/quantity</th>
<th>Elution volume</th>
<th>DNA conc.</th>
<th>A₂₆₀/A₂₈₀</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver</td>
<td>30 mg</td>
<td>200 μl</td>
<td>235.2 ng/μl</td>
<td>1.86</td>
<td>47 μg</td>
</tr>
<tr>
<td>Rat skeletal muscle</td>
<td>20 mg</td>
<td>200 μl</td>
<td>50.5 ng/μl</td>
<td>1.80</td>
<td>10.1 μg</td>
</tr>
<tr>
<td>Rat muscle</td>
<td>20 mg</td>
<td>200 μl</td>
<td>123.5 ng/μl</td>
<td>1.84</td>
<td>24.7 μg</td>
</tr>
<tr>
<td>Yellow adipose tissue</td>
<td>30 mg</td>
<td>200 μl</td>
<td>49 ng/μl</td>
<td>1.80</td>
<td>9.8 μg</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>30 mg</td>
<td>200 μl</td>
<td>116.7 ng/μl</td>
<td>1.88</td>
<td>23.3 μg</td>
</tr>
<tr>
<td>Rat kidney</td>
<td>20 mg</td>
<td>200 μl</td>
<td>183.4 ng/μl</td>
<td>1.79</td>
<td>36.7 μg</td>
</tr>
<tr>
<td>HT29 cell culture</td>
<td>1x 10⁵</td>
<td>200 μl</td>
<td>51.5 ng/μl</td>
<td>1.81</td>
<td>10.3 μg</td>
</tr>
<tr>
<td>HCT116 cell culture</td>
<td>3x 10⁶</td>
<td>200 μl</td>
<td>60 ng/μl</td>
<td>1.71</td>
<td>12.0 μg</td>
</tr>
<tr>
<td>Rat brain</td>
<td>20 mg</td>
<td>200 μl</td>
<td>9.4 ng/μl</td>
<td>1.69</td>
<td>1.88 μg</td>
</tr>
<tr>
<td>Insects</td>
<td>2.7 mg</td>
<td>30 μl</td>
<td>12.9 ng/μl</td>
<td>1.65</td>
<td>0.39 μg</td>
</tr>
</tbody>
</table>

XIV. AVERAGE FRESH BIOLOGICAL MATERIAL PROCESSING TIMES

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

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K</td>
<td>H315, H319, H334, H335, P260, P305+P351+P338, P342+P311</td>
</tr>
<tr>
<td>RNAse A</td>
<td>H319, P260, P305+P351+P338, P342+P311, P304+P341, P302+P352</td>
</tr>
</tbody>
</table>

**H302** Harmful if swallowed. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **P260** Do not breathe dust/fume/gas/mist/vapours/spray. **P313** Get medical advice/attention. **P305 + P351 + P338** IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P342 + P311** Call a POISON CENTER or doctor/physician. **P304 + P341** IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. **P302 + P352** IF ON SKIN: Wash with plenty of soap and water.