

Article number:
11003314 (50 pcs.)
11003316 (250 pcs.)

Research use only

Version: 012018



Axonlab LifeScience
GENOMICS

extrAXON

DNA TISSUE KIT

Kit for DNA isolation from animal tissue and cell culture

The **extrAXON DNA TISSUE KIT** is designed for a 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.

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Instructions to use
Version 012018

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I. | Intended Use

II. | Components of the kit and storage conditions

Number of isolation	50 isolations	250 isolations	Storage Conditions
TL Buffer (Tissue Lysis Buffer)	19 ml	94 ml	15°C – 25°C
▶ Proteinase K* (lyophilized)	1 tube	5 tubes	- 20°C ¹
Proteinase Buffer	1.4 ml	7 ml	15°C – 25°C
▶ RNase** (lyophilized)	1 tube	5 tubes	- 20°C ²
RNase Buffer	220 µl	1.1 ml	15°C – 25°C
TB Buffer (conc.)*** (Binding Buffer)	10 ml	44 ml	15°C – 25°C
TW1 Buffer*** (Wash Buffer 1)	17 ml	82 ml	15°C – 25°C
TW2 Buffer (conc.)*** (Wash Buffer 2)	9 ml	41 ml	15°C – 25°C
Elution Buffer	10 ml	5 x 10 ml	15°C – 25°C
DNA Purification Columns	50 pcs	5 x 50 pcs	15°C – 25°C
Collection Tubes (2 ml)	50 pcs	5 x 50 pcs	15°C – 25°C

¹ After reconstitution, **Proteinase K** should be kept at - 20°C.

² 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 1.4 ml **Proteinase Buffer** to a tube containing **Proteinase K** lyophilizate

** Prior to the first use, add 220 µl **RNase Buffer** to a tube containing **RNase A** lyophilizate

*** Prior to the first use, add an appropriate amount of **96-100% ethanol** to **TB, TW1** and **TW2 Buffers** (see the instructions on the bottle label and in the table page 4). It is recommended to mark the bottle containing added alcohol.

Number of isolation	50 isolations	250 isolations
TB Buffer	10 ml	44 ml
96-100% ethanol	15 ml	66 ml
Total volume	25 ml	110 ml
TW1 Buffer	17 ml	82 ml
96-100% ethanol	17 ml	82 ml
Total volume	34 ml	164 ml
TW2 Buffer	9 ml	41 ml
96-100% ethanol	21 ml	96 ml
Total volume	30 ml	137 ml

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.

- 96 – 100% ethanol PFA
- 1.5 – 2 ml sterile microcentrifuge tubes
- automatic pipettes and sterile DNase-free tips
- disposable gloves
- microcentrifuge with rotor for 1.5 – 2 ml ($\geq 11\,000 \times g$)
- dry block heater or water bath (up to 55°C)
- vortex mixer

Might 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 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.

- saline – physiological fluids
 - 1M DTT – hair
- Preparation: dissolve 1.54 g DTT in 10 ml H_2O . Aliquot and store at -20°C.*

- scissors, scalpel
- 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)

III. | Additional materials and equipment required

IV. | Principle

DNA purification procedure consists of four steps and utilizes spin DNA Purification Columns with membranes which efficiently and selectively bind nucleic acids. In the first isolation step, tissue is homogenized in order to disintegrate intercellular bonds (epithelial tissue) and fragmentize high-molecular proteins (muscle or connective tissue). Then 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 RNase A. Homogenate is separated from undigested tissue remains by centrifugation and combined with chaotropic salts. Mixture is then applied to DNA Purification Column membrane and DNA is bound. A two-step washing stage effectively removes impurities and enzyme inhibitors. 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 **extrAXON DNA TISSUE KIT** is tested using 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
- ▶ cell culture: 10^3 – 10^7 cells
- ▶ physiological fluids (urine, PMR, peritoneal fluid): 1 – 5 ml
- ▶ hair: 10 – 30 mg
- ▶ insects: 1 – 30 mg
- ▶ rodent tail: up to 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)
- ▶ 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

- Tissue is treated as a biohazardous material for its potential pathogen content or health and life-threatening substances. While working with tissue, 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 glove and suitable lab coat at all times.
- The use of sterile filter tips is recommended.
- Avoid cross-contamination between sample preparations.
- Guanidine salts' can form highly reactive compounds when combined with bleach or other oxidation components. In case of spillage, clean the surface with suitable laboratory detergent and water.
- In case of spillage of liquid containing microorganisms, clean the contaminated surface with detergent-water solution.

DNA ELUTION

An optimal volume of Elution Buffer used should be chosen in line with the quantity of sample material and 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. 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.

If a high DNA concentration is desired, the elution volume may be reduced to 50 µl. However, it may reduce the efficiency of DNA retrieval. It is essential to apply Elution Buffer precisely to the centre of the membrane.

VII. | Safety Precautions

VIII. | Recommendations and important notes

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, 100 – 200 µl elution should be performed. For the second elution, repeat steps 15 – 18 of the Isolation Protocol (see section XI), placing the DNA 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 DNA Purification Column 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 3 of the Isolation Protocol, section XI).

FOAM FORMATION IN THE TL BUFFER

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

A. FRESH OR FROZEN SOLID TISSUE

Quantity: 1 – 30 mg;

Sample material: animal or human tissues.

IX. | Sample Preparation

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 (LN₂, CO₂)

1. Place tissue frozen in LN₂ or CO₂ 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 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 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.
3. Continue the isolation from step 1b 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 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. 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 TL 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 $3000 \times g$. If a compact cell pellet is not formed, wash the cells twice with **1 ml cold PBS buffer**.
2. Add **$375 \mu\text{l}$ 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. Should this happen, pipette carefully, using a $\geq 1000 \mu\text{l}$ 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. 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 TL Buffer**. Mix thoroughly by vortexing for 30 sec.
4. Continue the isolation from step 1b of the Isolation Protocol (section XI).

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 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 μ l TL Buffer**, **40 μ l 1M DTT** and **25 μ l 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 60 – 120 s from time to time. A thermomixer may be used.

4. After the lysis is complete, continue the isolation from step 2 of the Isolation Protocol (section XI).

▶ For small quantities of sample material, elution in 200 μ l 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 μ l 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 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).

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 60 s at 500 x g. Depending on the fragmentation' degree go to step 1b 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 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 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' 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).
 - ▶ For small quantities of sample material, elution in 200 µl Elution Buffer, followed by precipitation, is recommended.

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. Unless otherwise stated, conduct all the isolation steps at room temperature.

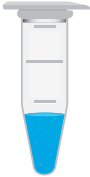
X. | Prior to isolation

XI. | Isolation Protocol

+ 375 μ l
TL Buffer

+ 25 μ l
Proteinase K

55°C
30-60 min.



STEP 1

- Place the fragmented biological material in a 2 ml tube. Add **375 μ l TL Buffer** and vortex for 20 s.
 - ▶ If a thick foam occurs, centrifuge the sample at 11000 x g for 60 – 120 s. Refer to section VIII. Recommendations and Important Notes.
- Add **25 μ l 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.
- If the RNase A is to be used, add **4 μ l RNase A** and incubate at **37°C** for 5 min.

STEP 2

Add **400 μ l TB Buffer** and mix thoroughly for 10 s.

Centrifuge for 120 s at **11000 – 21000 x g**.

Transfer the supernatant into a purification minicolumn placed in a Collection Tube. Ensure no tissue remains are transferred along with it.

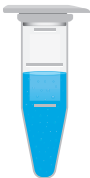
- ▶ For homogenization with the use of bead-beating tubes: carefully pipet the appropriate volume of the supernatant by placing a 200 μ l pipette tip (N.B.: a 1 ml tip may become clogged by the beads) into the filling. Tissue remains should either lie on one side of the tube or at the bottom.

Centrifuge for 60 s at **11000 – 15000 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 the purification column to a new 2 ml Collection Tube.


11-21000 x g




120 s


11-15000 x g




60 s

STEP 3

Add **600 µl TW1 Buffer** and centrifuge for 30 s at **11000 – 15000 x g**. Discard the filtrate and reuse the Collection Tube.

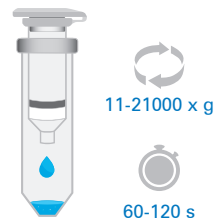
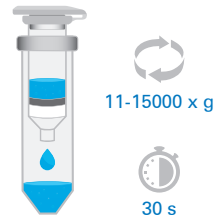
Add **500 µl TW2 Buffer** and centrifuge for 30 s at **11000 – 15000 x g**.

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



STEP 4

Add **100-200 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 the minicolumn at room temperature for 120 s.

Centrifuge at **11000 – 15000 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
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 TL 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.
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 TL Buffer. The tissue must first be fragmented into the smallest possible pieces and homogenized by an appropriate method.
	Incomplete tissue lysis.	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 TL Buffer for 16 h.
	Reduced Proteinase K activity.	Inappropriate storage conditions for the Proteinase K. When applicable, ensure that the sample material is thoroughly washed with PBS buffer (see section IX B, C, E and H).
	DNA purification column has become clogged.	See «Column becomes clogged during purification».
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to the membrane, heat it to 80°C. Apply Elution Buffer directly to the centre of the membrane. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase 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.



Problem	Possible cause	Solution
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 the Proteinase K in TL Buffer for 16 h until the lysate is clear.
	Reduced Proteinase K activity.	Inappropriate storage conditions for Proteinase K. When applicable, ensure that the sample material is thoroughly washed with PBS buffer (see section IX B, C, E and H).
Purified DNA is degraded.	Old or damaged material was used.	Performing an isolation from fresh or properly preserved tissues is recommended.
	Inappropriate tissue storage conditions or improper preservation.	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.
	The DNA degraded as a result of overintensive homogenization.	The recommended homogenization conditions should be applied (see section IXA).
RNA contamination present.	Tissue containing much RNA.	Perform digestion with RNase A (step 4 of the Isolation Protocol).
Inhibitor of downstream enzymatic reactions.	Purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual TW2 Buffer is left in the DNA purification column after centrifugation in step 13.

XIII. | 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	1 x 10 ⁵	200 µl	51.5 ng/µl	1.81	10.3 µg
HCT116 cell culture	3 x 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. | Average fresh biological material processing time

Sample Material	Digestion in a block heater, no homogenization, periodical mixing	Digestion in a thermomixer	Liquid nitrogen homogenization	Approximate lysis time
Rat liver	3 – 6 h	2 – 4 h	1 – 2 h	min 1 h, max 6 h
Rat skeletal muscle	2 – 3 h	2 h	≤ 1 h	min 0.5 h, max 3 h
Rat heart	2 – 3 h	1 – 1.5 h	≤ 1 h	min 0.5 h, max 3 h
Yellow adipose tissue	1 – 1.5 h	1 h	0.5 h	min 0.5 h, max 1.5 h
Brown adipose tissue	1 – 1.5 h	1 h	0.5 h	min 0.5 h, max 1.5 h
Rat kidney	1.5 – 3 h	0.5 – 2 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	min 5 min, max 1 h
HCT116 cell culture	0.5 – 1 h	5 – 30 min	No data	min 5 min, max 1 h
Rat brain	0.5 – 1 h	0.5 – 1 h	No data	min 0.5 h, max 1 h
Insects	No data	1 – 2 h	0.5 – 2 h	min 0.5 h, max 2 h

XV. | Safety information

extrAXON

DNA TISSUE KIT



Proteinase K (lyophilized)

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



TL Buffer

H319
P264, P305+P351+P338



TB Buffer

H318, H332, H315, H412
P280, P261, P305+P351+P338
P301+P312 P330, P304+340 P312



TW1 Buffer

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

Danger

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.

