A&A BIOTECHNOLOGY
innovating life science

Genomic Micro AX Tissue Gravity

Gravity flow kit for genomic DNA purification from tissue and cell culture.
version 1220

100 isolations

Cat. # 104-100

gravity flow

The binding capacity of the genomic DNA purification column is 20 µg of DNA.

For R&D use only

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Kit Contents

Component	Quantity	Store at
Micro AXD columns (DNA binding capacity – 20 µg)	100 pcs	+4 to +8 °C
Gravity tubes	100 pcs	Room Temp.
LSU lysis buffer	50 ml	Room Temp.
K1G equilibrating solution	60 ml	Room Temp.
W1G first wash solution	70 ml	Room Temp.
W2 second wash solution	60 ml	Room Temp.
E elution buffer	20 ml	+4 to +8 °C
N neutralizing buffer	1 ml	Room Temp.
Proteinase K	2 x 1.1 ml	+4 to +8 °C
T solution	400 µl	+4 to +8 °C

Equipment and materials necessary for DNA isolation that are not included in kit

1. Material for DNA isolation
2. DTT (dithiothreitol) for isolation DNA from semen (cat. # 2010-5, 2010-25)
3. 1.5 ml sterile Eppendorf tubes
4. Clear PCR tubes (option)
5. Heatblock or incubator set to 50 °C
6. Vortex
7. Benchtop microcentrifuge (option)

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Isolation protocol

1. Samples:
   A. Solid tissues:
   up to 10 mg of fragmented tissues transfer to Eppendorf tubes (not included);
   B. Cell cultures:
   0,5 x 10^6 of cell culture centrifuge and discard the supernatant;
   C. Semen:
   up to 10 µl of semen transfer to Eppendorf tubes and add 20 µl 1M DTT (not included, cat. # 2010-5, 2010-25).

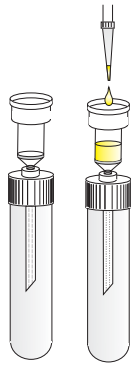
2. Add 400 µl of LSU lysis buffer and 20 µl of Proteinase K.

3. Mix by vortexing and incubate at 50 °C:
   cells: for 10 min;
   solid tissues: for about 1-2 h until complete digestion.
   Mix samples from time to time by vortexing.

   The incubation step can be performed in Eppendorf Thermomixer or analogous equipment at 1400 RPM and 50 °C.

   RNA digestion (optional): See „Additional information" - page 5.

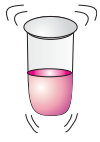
4. During incubation prepare the appropriate number of Micro AXD columns. Place each Micro AXD column tip into the fitting on top of the gravity tube cap. Place assembled Micro AXD columns with tubes in a suitable rack.



   Subsequently apply 500 µl of K1G equilibrating solution onto each Micro AXD column.

   The solution should penetrate the column and start dripping down at the bottom fo micro-drain by means of gravity. As soon as the solution stops dripping the Micro AXD column is ready for the DNA purification process.

5. Vortex the lysed samples intensely for 2 min at 1000 -1500 RPM after the incubation step.



   This is the key step for efficiency of DNA isolation.

6. Centrifuge for 5 min at 10 000–14 000 RPM.

7. Apply the samples onto pre-equilibrated Micro AXD columns.

Wait until the lysates pass through the columns by gravity. This takes up to 10 min.

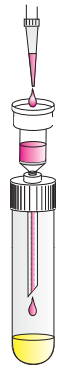
The flow rate strongly depends on DNA concentration in the sample.

As soon as the lysate stops dripping proceed to the next step.

8. Add 600 µl of W1G first wash solution.

Wait until the W1G first wash solution passes through the Micro AXD columns.

9. Add 500 µl of W2 second wash solution.



Wait until the W2 second wash solution passes through the Micro AXD columns.

10. Add 60 µl of E elution buffer and wait 5 min.

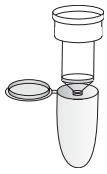
The purpose of this step is to decrease the total volume of eluate, since the column void volume is about 60 µl.

E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use.

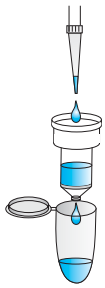
11. Prepare the 1.5 ml elution tubes (not included) and add 5 µl of N neutralizing buffer to the bottom of each tube.

DNA neutralization: See “Additional information” – page 5.

12. Transfer the Micro AXD columns to the prepared elution tubes.



13. Elute the DNA by adding 120 µl of E elution buffer onto the Micro AXD columns. Wait 10 min to allow E elution buffer to pass through the Micro AXD columns.



The lack of elution it means a very high DNA concentration in a sample. In which case we recommend the centrifugation the sample (elution tube with Micro AXD column together) for 30 s – 1 min at 5000 RPM.

E elution buffer loses activity after prolonged contact with air. Always close the E elution buffer vial tightly directly after use.

14. Discard the Micro AXD columns. Close the tubes with purified DNA.

Additional Information

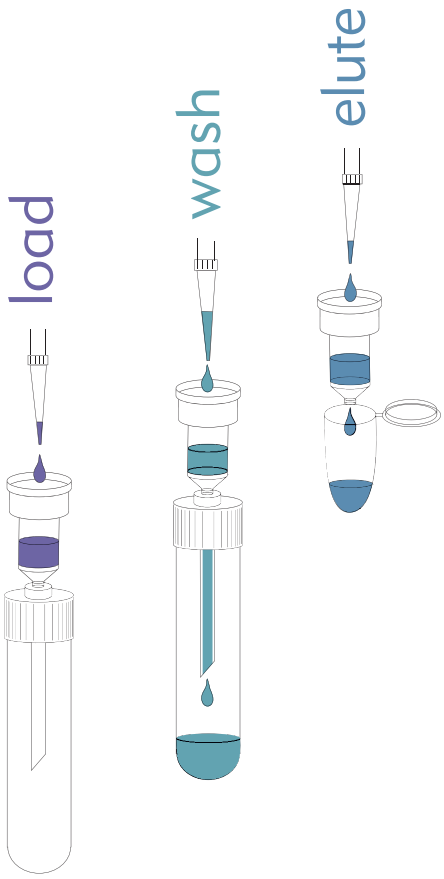
RNA digestion. Add 5 µl of RNAse (10 mg/ml solution) (not included, cat. # 1006-10, 1006-50) and mix sample by vigorous vortexing for 20 s. Incubate the sample for 5 min at room temp.

DNA neutralization. The E elution buffer is strongly alkaline and may cause DNA degradation upon freezing. Thus it is necessary to use N neutralizing buffer. We recommend to add the N neutralizing buffer to the elution tube before the elution step point 11. of isolation protocol).
If the N neutralizing buffer was not added in point 11. of isolation protocol, it can be added directly before freezing DNA samples.
The use of N neutralizing buffer enables secure DNA storage conditions at 10 mM TrisHCl, pH 8.5.

Notes

Problem	Reason	Solution
Very slow flow rate of lysate through column.	Highly concentrated DNA in sample.	Place microcolumn in an Eppendorf tube and spin it. At the next isolation reduce the quantity of original sample.
Air bubbles present in the receiving tube capillary.	The gravity microcolumn is not attached tightly to the receiving tube.	Reattach the column in luer-like fitting simultaneous by pressing the column down and twisting.

Gravity flow technology



Products based on Gravity flow technology

Content	Quantity	Material	Cat. #
Plasmid Mini AX Gravity	100 isolations	Plasmids	015-100
Genomic Micro AX Swab Gravity	100 isolations	Swabs	105-100
Genomic Micro AX Swab Gravity Plus	100 isolations + swab tools	Swabs	105-100P
Genomic Micro AX Bacteria Gravity	100 isolations	Bacteria	102-100
Genomic Micro AX Bacteria+ Gravity	100 isolations	G+ Bacteria	102-100M
Genomic Micro AX Plant Gravity	100 isolations	Plant	103-100
Genomic Micro AX Blood Gravity	100 isolations	Blood	101-100
Bead-Beat Micro AX Gravity	20 isolations	Difficult samples	106-20
	100 isolations	Difficult samples	106-100

Buffer E functionality test

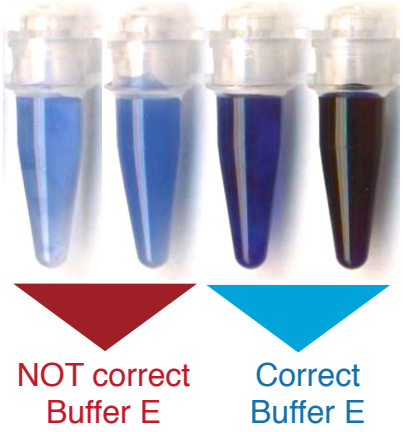
Buffer E has a critical influence on DNA elution efficiency and thus overall DNA purification yield. The kit contains solution T which enables testing of the elution buffer E correct functionality.

Typically it is suggested to perform such a test in the following cases:

- the buffer E was not used for a long period of time (at least 2 months)
- the buffer E vial was stored at room temperature for a long period of time (at least 2 weeks)
- the buffer E vial was not closed tightly

Testing the elution buffer E functionality procedure

1. Transfer 20 µl of elution buffer E to a clear 200 µl PCR tube
2. Add 2 µl of solution T and mix the sample
3. Wait 2 min and compare the mixture colour with the reference colour guide



Ordering information

Product	Quantity	Cat. #
Proteinase K solution (20 mg/ml)	1 ml	1019-20
Proteinase K lyophilized	25 mg	1019-25L
	100 mg	1019-100L
	250 mg	1019-250L
	1000 mg	1019-1L
RNAse solution (10 mg/ml)	1 ml	1006-10
	5 ml	1006-50
DTT (dithiothreitol)	5 g	2010-5
	25 g	2010-25

Safety information

DANGER

Proteinase K

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
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.

WARNING

LSU lysis buffer

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

E elution buffer

H314 Causes severe skin burns and eye damage.
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.
P310 Immediately call a Poison Center or doctor/physician.