A&A BIOTECHNOLOGY
innovating life science

Genomic Midi AX Direct

Versatile, increased efficiency kit for genomic DNA purification from various sources.
Procedure without DNA precipitation.

version 0517

20 isolations

Cat. # 895-20D

The binding capacity of the DNA purification column is 100 µg of DNA.

For R&D use only.

A&A BIOTECHNOLOGY, Aleja Zwycięstwa 96/98, 81-451 Gdynia, Poland
tel: +48 58 6982194, +48 883 323 761, fax: +48 58 6228578
VAT PL5840254403
info@aabiot.com www.aabiot.com

Kit Contents

Component	Quantity	Store at
Spin 100 AX columns	20 pcs	+4 to +8 °C
15 ml tubes	40 szt.	Room Temp.
Counterweight cartridge	1 pcs	Room Temp.
L1.4 lysis solution	50 ml	Room Temp.
W1G first wash buffer	70 ml	Room Temp.
W2 second wash buffer	40 ml	Room Temp.
TE buffer	50 ml	Room Temp.
E elution buffer	15 ml	+4 to +8 °C
Proteinase K	2 x 1.1 ml	+4 to +8 °C
N neutralizing buffer	1 ml	Room Temp.
T solution	400 µl	+4 to +8 °C

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

1. Material for DNA isolation
2. Enzymes (option – depending on type of biological material):
   Lysosyme – 10 mg/ml, 400 U (cat. # 1005-10, 1005-50)
   Lysostaphin – 0.4 U/µl (cat. # 1007-400, 1007-2000)
   Mutanolysin – 10 U/µl (cat. # 1017-5, 1017-10, 1017-50)
3. RNAse (cat. # 1006-10, 1006-50) (optional)
4. 1.5 ml Eppendorf tubes
5. 15 ml Falcon tubes
6. Thermoblock or incubator set to 37 °C, 50 °C
7. Centrifuge with swing-out rotor for 15 ml tubes (Falcon type)

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

Material preparation

Fresh or frozen blood samples

1. Transfer 2 ml of blood sample to a 15 ml tube (not included).
   If the sample volume is less than 2 ml add appropriate volume of TE buffer to reach the final volume of 2 ml.
2. Add 2 ml of L1.4 lysis solution and 100 µl of Proteinase K solution.
3. Mix thoroughly and incubate for 20 min at 50 °C.
   (**ATTENTION**: Do not prolong incubation time)
4. Mix the sample intensely by vigorous vortexing for 20 s.
5. Follow point 1. of the protocol.

Bacteria

1. Transfer 1–5 ml of bacterial culture to a 15 ml tube (not included).
2. Centrifuge and discard the supernatant.
3. Suspend the bacterial pellet in 2 ml of TE buffer.
   Add 20 µl of lysozyme solution to final concentration 10 mg/ml (not included, cat # 1005-10, 1005-50) and incubate for 15 min at 37 °C.
   ATTENTION: enzyme of choice:
   for *S.aureus* – we recommend using lysostaphin (cat. # 1007-400, 1007-2000)
   for *Streptococcus, Lactobacillus, Lactococcus, Listeria* – we recommend using mutanolysin (cat. # 1017-5, 1017-10, 1017-50) or lysozyme with mutanolysin.
4. Add 2 ml of L1.4 lysis solution and 100 µl of Proteinase K solution. Mix the sample by inverting the tube and incubate at 50 °C until mixture is completely clear (usually 60 min).
   RNA digestion (optional): Add 10 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 in room temp.
5. Follow point 1. of the protocol.

Tissues

1. Cut 50–100 mg of tissue into small pieces and/or grind in sterile mortar under liquid nitrogen, until it is completely powdered. Wait until nitrogen evaporates and transfer tissue powder to a 15 ml tube (not included).
2. Add 2 ml of TE buffer, 2 ml of L1.4 lysis solution and 100 µl of Proteinase K solution.
3. Mix thoroughly and incubate at 50 °C until complete tissue digestion (usually 120-240 min). Mix the sample from time to time by vortexing.
   RNA digestion (optional): Add 10 µl of RNAse (10 mg/ml solution) (not included, cat. # 1006-10, 1006-50) and mix sample by vigorous vortexing for 20 s. Incubate he sample for 5 min in room temp.
4. Centrifuge for 10 min at 4000–5000 x g. Transfer the supernatant to new 15 ml tube.
5. Follow point 1. of the protocol.

Tissue Cell culture

1. Transfer 1 x 10^7 of tissue cell culture to a 15 ml tube (not included).
2. Centrifuge and discard the supernatant.
3. Suspend pellet in 2 ml of TE buffer.
4. Add 2 ml of L1.4 lysis solution and 100 µl of Proteinase K solution. Mix the sample by inverting the tube and incubate for 30 min at 50 °C.

   RNA digestion (optional): Add 10 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 or 5 min at room temp.
5. Centrifuge for 10 min at 4000-5000 x g. Transfer the supernatant to new 15 ml tube.
6. Follow point 1. of the protocol.

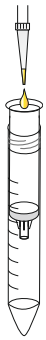
Isolation protocol

Attention:

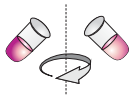
E elution buffer loses activity upon prolonged contact with air. Always close the tube with E elution buffer vial tightly directly after use.

1. Apply the samples onto the Spin 100AX columns placed inside 15 ml tubes.

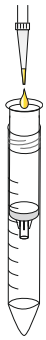
   If you have an odd number of samples, please remember about counterweight cartridge before centrifugation.



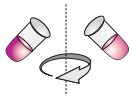
2. Centrifuge in a swing-out rotor for 2 min at 3000 RPM.



3. Transfer the Spin 100AX columns to new 15 ml tubes (included). Apply 3 ml of W1G first wash solution.

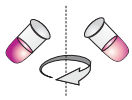


4. Centrifuge for 2 min at 3000 RPM.



5. Add 1.5 ml of W2 second wash solution.

6. Centrifuge for 2 min at 3000 RPM.

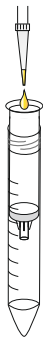


7. Prepare 15 ml elution tubes (included) and add to the bottom of each tube 10 µl of N neutralizing buffer.

See "DNA neutralization" – page 6.

8. Before using E elution buffer we recommend to perform the functionality test. See "E elution buffer functionality test" – page 6.

Place the Spin 100AX columns into the elution tubes prepare on point 7.
Add 400 µl of E elution buffer to Spin 100AX columns.

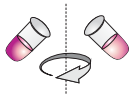


E elution buffer loses activity upon prolonged contact with air. Always close the tube with E elution buffer vial tightly directly after use.

E elution buffer must be stored at +4 to +8 °C.

9. Incubate for 2 min at room temp.

10. Centrifuge for 2 min at 3000 RPM.



11. Discard the the Spin 100AX columns. Close the elution tubes containing purified DNA.

Store the purified DNA at +4 ºC or –20 ºC.

DNA neutralization

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 N neutralizing buffer to the elution tube before the elution step (point 7. of isolation protocol).
If the N neutralizing buffer was not added in point 7. 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.

DNA can be directly use used for sequencing, PCR, cloning, etc.

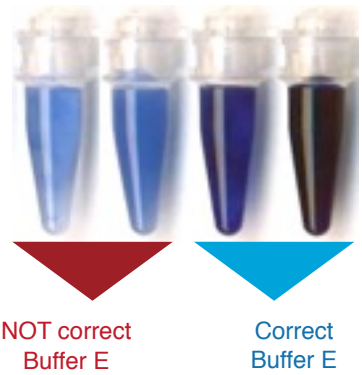
E elution buffer functionality test

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

Typically it is suggested to perform such test in the following cases:
- the E elution buffer was not used for a long period of time (at least 2 months)
- the E elution buffer vial was stored at room temperature for a long period of time (at least 2 weeks)
- the E elution buffer vial was not closed tightly

Testing the E elution buffer functionality procedure

1. Transfer 20 µl of E elution buffer to a clear 200 µl PCR tube
2. Add 2 µl of T solution 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)	1.1 ml	1019-20
Proteinase K lyophilized powder	25 mg	1019-25L
	100 mg	1019-100L
	250 mg	1019-250L
	1000 mg	1019-1L
TE buffer	100 ml	297-100
RNAse (DNAse free) solution (10 mg/ml)	1 ml	1006-10
	5 ml	1006-50
Lysosyme solution (10 mg/ml, 400 U/µl)	1 ml	1005-10
	5 ml	1005-50
Lysostaphin solution (1 mg/ml, 0.4 U/µl)	400 U	1007-400
	2000 U	1007-2000
Mutanolysin solution (10 U/µl)	5 000 U	1017-5
	10 000 U	1017-10
	50 000 U	1017-50

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

L1.4 lysis solution
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.

WARNING

W1G first wash solution
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.