A&A BIOTECHNOLOGY
innovating life science

Genomic Maxi AX

Versatile, increased efficiency kit for genomic DNA purification from various sources.

version 0517

10 isolations

Cat. # 995-10

The binding capacity of the DNA purification column is 500 µg of DNA.

For R&D use only

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Kit Contents

Component	Quantity	Store at
Spin 500 AX columns	10 pcs	+4 to +8 °C
50 ml tubes	20 pcs	Room Temp.
Counterweight column	1 pcs	Room Temp.
L1.4 lysis solution	55 ml	Room Temp.
K2 wash solution	120 ml	Room Temp.
K3 elution solution	55 ml	Room Temp.
TE buffer	80 ml	Room Temp.
Isopropanol	45 ml	Room Temp.
Proteinase K	2 x 1.1 ml	+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):
   Lysozyme – 10 mg/ml, 400 U/µl (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 – 10 mg/ml (cat. # 1006-10, 1006-50) (optional)
4. Sterile water (nuclease free, DEPC treated) (cat. # 003-075, 003-25) (optional)
5. 70% ethanol
6. 50 ml Falcon tubes
7. Vortex
8. Centrifuge with swing-out rotor for 50 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 5 ml of blood sample to a 50 ml tube (not included).
   If the sample volume is less than 5 ml add appropriate volume of TE buffer to reach the final volume of 5 ml.
2. Add 5 ml of L1.4 lysis solution and 200 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 5–20 ml of bacterial culture to a 50 ml tube (not included).
2. Centrifuge and discard the supernatant.
3. Suspend the bacterial pellet in 5 ml of TE buffer.
   Add 50 µl of lysozyme solution (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 5 ml of L1.4 lysis solution and 200 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 at room temp.
5. Follow point 1. of the protocol.

Tissues

1. Cut 150–300 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 50 ml tube (not included).
2. Add 5 ml of TE buffer, 5 ml of L1.4 lysis solution and 200 µl of Proteinase K solution.
3. Mix thoroughly and incubate at 50 °C until complete tissue digestion (usually 120–240 min). Mix 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 the sample for 5 min at room temp.
4. Centrifuge for 10 min at 4000–5000 x g. Transfer the supernatant to a new 50 ml tube.
5. Follow point 1. of the protocol.

Tissue cell culture

1. Transfer 1.5 x 10^6–3 x 10^7 of tissue cell culture to a 50 ml tube (not included).
2. Centrifuge and discard the supernatant.
3. Suspend pellet in 5 ml of TE buffer.
4. Add 5 ml of L1.4 lysis solution and 200 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 for 5 min at room temp.
5. Centrifuge for 10 min at 4000–5000 x g. Transfer the supernatant to a new 50 ml tube.
6. Follow point 1. of the protocol.

Isolation protocol

1. Apply the samples onto the Spin 500AX columns placed inside 50 ml tubes.

   If you have odd number of samples, please remember about counterweight columns before centrifugation

2. Centrifuge in a swing-out rotor for 2 min at 4000 RPM.

3. Transfer the Spin 500AX columns to new 50 ml tubes (included). Add 5 ml of K2 wash solution.

4. Centrifuge for 2 min at 4000 RPM.

5. Add 5 ml of K2 wash solution.

6. Centrifuge for 2 min at 4000 RPM.

7. Transfer the Spin 500AX columns to new 50 ml tubes (included). Add 2,5 ml of K3 elution solution.

8. Incubate for 2 min at room temp.

9. Centrifuge for 1 min at 4000 RPM.

10. Add 2.5 ml of K3 elution solution.

11. Centrifuge for 1 min at 4000 RPM.

12. Remove the Spin 500AX columns. The approximate eluate volume should be ~5 ml.
Add 4 ml of isopropanol to the eluate. Close the tubes with caps and mix carefully by inverting the tubes a few times.

If white precipitate is present in the tubes, please proceed to point A.
If white precipitate is not present in the tubes, please proceed to point B.

A. The white precipitate is visible

1. Centrifuge the samples for 2 min at 4000 RPM.

2. Carefully discard supernatants and wash the pellets with 2 ml of 70% ethanol (not included).

3. Centrifuge for 2 min at 4000 RPM.

4. Carefully discard supernatants and air-dry the pellets for 10 min at room temp. by placing the tubes up-side-down.

5. Dissolve the DNA pellets in a desired volume of TE buffer (included) or sterile nuclease-free water (not included).

To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.

Store the purified DNA at +4 °C or -20 °C.

B. The white precipitate is not visible

1. Transfer the samples to new centrifuge tubes suitable for high centrifuge speed.
   Centrifuge the samples for 30 min at 15 000 x g.

2. Carefully discard supernatants and wash the pellets with 2 ml of 70% ethanol (not included).

3. Centrifuge for 5 min at 15 000 x g.

4. Carefully discard supernatants and air-dry the pellets for 10 min at room temp. by placing the tubes up-side-down.

5. Dissolve the DNA pellets in a desired volume of TE buffer (included) or sterile nuclease-free water (not included).

   To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.

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

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 (400 U/µl)	1 ml	1005-10
	5 ml	1005-50
Lysostaphin solution (0,4 U/µl)	400 U	1007-400
	2 000 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.

DANGER

K2 wash solution

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
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

K3 elution solution

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
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

Isopropanol

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.