A&A BIOTECHNOLOGY
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Manual

MagnifiQ™ 96 Genomic DNA instant kit

Kit for automated, magnetic isolation of DNA in the 96 samples per plate format. Contains ready-to-use, reagent-filled plates and all necessary consumables.

catalog #	size	compatible devices *
604A-96V-960	960 isolations	Auto-Pure 96

*** Compatible devices**
The kit has been tested with ThermoFisher Scientific KingFisher Flex and Allsheng Auto Pure 96 devices. This does not preclude it from working with other devices. If your device is not listed, please contact us at info@aabiot.com.

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Advantages

- MagnifiQ™ 96 Genomic DNA instant kit does not require initial preparation of buffers. Just add samples to the plate and get extracted material within approximately half an hour.
- Enables isolation of different samples with universal kit and automated extraction programme.

Sample type

	sample size
Bacteria G-, G+ (cultures)	up to 2 x 10^8
Yeast (cultures)	up to 1 ml
Cell cultures	up to 1 x 10^6
Blood fresh or frozen, serum, plasma	up to 200 µl
Animal tissue	up to 20 mg
Swab	1 pc
Feces	20 - 50 mg
Feces (sample stored in conservation solution)	250 - 500 µl

Specification

protocol time	~ 30 min.
elution volume	100 µl [1]
elution solution	Tris buffer
binding capacity	30 µg DNA
downstream applications	qPCR, RT-qPCR, sequencing

[1] The elution volume prepared on the plate is 100 µl. To obtain a smaller elution volume, subtract the appropriate amount of elution solution from the wells on the EP plate. Attention! Do not reduce the elution volume below 50 µl. To obtain a larger elution volume, add the appropriate amount of elution solution from the wells on the EP plate. Attention! Do not increase the elution volume above 300 µl.

Description

MagnifiQ™ 96 Genomic DNA instant kit is designed for DNA isolation from various types of biological materials. Kit allows isolation of 96 samples per single extraction run. The isolated material is perfect for further analyzes and tests by qPCR and RT-PCR methods and for sequencing.

The **MagnifiQ™** product series is based on the automated isolation of nucleic acids with use of magnetic beads. This method significantly shortens working time and reduces risk of mistake in comparison to manual methods.

Contents

604A-96V-960

component	quantity	cat #	storage
CP - comb plate	1 pcs	K-P96V22C	15–25 °C
SP-D - sample plate	10 pcs	K-P96V22SAD	15–25 °C
WP 1 - wash 1 plate	10 pcs	K-P96V22W1A	15–25 °C
WP 2-3 - wash 2-3 plate	20 pcs	K-P96V22W23A	15–25 °C
EP - elution plate	10 pcs	K-P96V05EA	15–25 °C
Proteinase K	22 ml	K-PRK-22	4–8 °C*
LTE 2X buffer	210 ml	K-LTE2X-210	15–25 °C
Tris buffer	425 ml	K-TRIS-425	15–25 °C
LSDE buffer	530 ml	K-LSDE-530	15–25 °C
tip comb 96	5 x 2 pcs	K-C96V-2	15–25 °C
protective film	40 pcs	K-MQF-10	15–25 °C

* Proteinase K can be stored at 15-25 °C for up to 12 months.

Additional equipment and reagents

Necessary

- 96 deep-well plates 2.2 ml (sample lysis)
- automated pipette
- pipette tips
- centrifuge with swing-out rotor for 96 deep-well plates

Optional

- RNAse (10 µl per sample), cat # 1006-10
- 1.5 ml Eppendorf tubes
- thermoblock

Important notes

The following material preparation protocols apply to the procedure carried out in a 96 deep-well plate. If the material preparation is to be carried out in 1.5 ml Eppendorf tubes see the Additional Information.
In the case of isolation from feces due to the risk of contamination and lysis using a mixture of beads, the material preparation protocol refers to the procedure carried out in 2 ml screwed tubes containing a mixture of beads.

Material preparation

Bacteria G-, G+ (cultures)

Additional reagents you will need:

- **BacBreaker** bacteria lysis enzyme mix (20 µl per sample), cat # K-BACB-15A
- **BS** suspension buffer (200 µl per sample), cat # K-BS-250

Option:

- **Lysostaphin** (5 µl per sample), cat # 1007-3; For *Staphylococcus aureus* we recommend using lysostaphin.

1. Transfer the bacterial culture samples containing **2 x 10^8** bacteria to the 96 deep-well plate (not included).
   Seal the plate with a protective film and centrifuge for **10 min** at **1000 x g**.
   Remove the protective film.
   Carefully discard the supernatant with a pipette.
2. Suspend the bacterial pellet in **200 µl** of **BS** buffer.
3. Add **20 µl BacBreaker** enzyme mix to the wells.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10).
   Attention. For lysis of *Staphylococcus* bacteria, add **5 µl** of **lysostaphin**.
4. Mix the contents of the wells by pipetting.
   Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**
   Remove the protective film.

   Attention. For lysis of *Staphylococcus* bacteria with lysostaphin, mix and incubate for 20 min at 42 °C.
5. Add **200 µl LTE 2X** and **20 µl Proteinase K** to the wells.
   Mix the contents of the wells by pipetting.
6. Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**
7. Centrifuge the sample for **10 min** at **1000 x g**.

8. **Attention.** In the isolation protocol, use the supernatant as the sample.

   Follow point 1. of the protocol.

Yeast (cultures)

Additional reagents you will need:

- **Liticase** (10 µl per sample), cat # 1018-10
- **DTT RTU** (10 µl 1M solution per sample), cat # 2010-10P
- **BS** suspension buffer (200 µl per sample), cat # K-BS-250

Prepare **1M DTT** solution. Add 1 ml of sterile water (not included) to a vial containing DTT powder to obtain 1M DTT solution. Mix or vortex until complete dissolution of DTT powder. Store a clear solution at -20 °C.

1. Transfer **1 ml** of yeast culture to the 96 deep-well plate (not included).
   Seal the plate with a protective film and centrifuge for **10 min** at **1000 x g**.
   Remove the protective film.
   Carefully discard the supernatant with a pipette.

2. Suspend the yeast pellet in **200 µl** of **BS** buffer.

3. Add **10 µl lyticase** and **10 µl 1M DTT** to the wells.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10).

4. Mix the contents of the wells by pipetting.
   Seal the plate with a protective film and incubate for **20 min** at **37°C** with mixing **1600 RPM.**
   Remove the protective film.

5. Add **200 µl LTE 2X** and **20 µl Proteinase K** to the wells.
   Mix the contents of the wells by pipetting.

6. Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**

7. Centrifuge the sample for **10 min** at **1000 x g**.

8. **Attention.** In the isolation protocol, use the supernatant as the sample.

   Follow point 1. of the protocol.

Cell cultures

1. Transfer the cell culture samples containing **1 x 10^6** cells to the 96 deep-well plate (not included).
   Seal the plate with a protective film and centrifuge for **10 min** at **1000 x g**.
   Remove the protective film.
   Discard the supernatant with pipette.
2. Suspend the cell pellet in **200 µl** of **Tris** buffer.
3. Add **200 µl LTE 2X** and **20 µl Proteinase K** to the wells.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10).
4. Mix the contents of the wells by pipetting.
   Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**
5. Follow point 1. of the protocol.

Blood: fresh or frozen, plasma, serum

1. Transfer **200 µl** the sample to the 96 deep-well plate (not included).
2. Add **200 µl LTE 2X** and **20 µl Proteinase K** to the wells.
   Mix the contents of the wells by pipetting.
3. Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**
4. Centrifuge for **1 min** at **1000 x g**.

   Note. Centrifuge to remove remaining material from lids of the tubes and placement non-lysed material at the bottom of the plate.
5. Follow point 1. of the protocol.

Animal tissue

1. Transfer **up to 20 mg** of fragmented animal tissue to the 96 deep-well plate (not included).

   Note. The tissue should be fragmented by cutting into pieces or homogenization.

2. Add **400 µl LSDE** buffer and **40 µl Proteinase K** to the wells.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10).

3. Mix the contents of the wells by pipetting.
   Seal the plate with a protective film and incubate until complete lysis at **55 °C** with mixing **1600 RPM.**

   Information. The lysis step can last from 1 to 12 hours. For maximum efficiency, lysis should be carried out until the tissue is completely dissolved in the lysis solution.

4. Centrifuge for **10 min** at **1000 x g**.

5. **Attention.** In the isolation protocol, use the supernatant as the sample.

   Follow point 1. of the protocol.

Swabs with transport medium

No additional material preparation is required.

Dry swabs

1. Break or cut off part of the swab with the collected sample and place it in the wells of 96 deep-well plate (not included).

2. Add **500 µl** of **LSDE** buffer and **20 µl Proteinase K** to the wells.

   Note. Part of the swab with the sample should be completely immersed in the buffer.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10).

3. Mix the contents of the wells by pipetting.
   Seal the plate with a protective film and incubate for **20 min** at **55 °C** with mixing **1600 RPM.**

4. **Attention.** For the isolation process, take the entire volume of the sample, but no more than 400 µl.

   Follow point 1. of the protocol.

Feces (microbiome including G+, G- bacteria)

Additional reagents you will need:

- **Bead-beat tubes** (2 ml screwed Bead-beat tubes containing beads mix), cat # K-PKCM-50
- **L3P** precipitation solution (100 µl per sample), cat # K-L3P-60
- **LSDE** buffer (additional 500 µl per sample), cat # K-LSDE-500
- **antifoam** (10 µl per sample), cat# K-AYS-1

Before starting the process, mix the **LSDE** buffer with **antifoam**. Prepare the **LSDE-antifoam** mix by combining **1 ml** of **LSDE** buffer with **10 µl** of **antifoam** per sample. Prepare a volume sufficient for the number of isolated samples with a 10% excess. Mix well before use.

1. Transfer **20-50 mg** of stool sample to the 2 ml screwed Bead-beat tube containing beads mix.
   Add **1 ml** of **LSDE-antifoam** buffer.

2. **Option A:** Bead Beating. Use the suitable tube bead beater with the following programme:
   3 x run of 20 s at **maximum force** with **2 min** cool down rest.

   Option B: Place the tubes in the incubator with mixing function (e.g. Thermomixer) and vortex at **2000 RPM** for **30 min** at **room temperature**.

3. Centrifuge for **5 min** at **10 000 RPM**.

4. Transfer **500 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

5. Add **20 µl Proteinase K.**

6. Vortex the sample for **10 s** and incubate for **15 min** at **50 °C** with shaking **1400 RPM**.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10) and incubate for **10 min** at **37 °C** with shaking at **1400 RPM.**

7. Add **100 µl** of **L3P** precipitation solution. Close the tube and mix whole content by inverting the tube.

8. Place on ice for **3 min.**

9. Centrifuge the sample for **5 min** at **10 000 RPM**.

10. **Attention.** In the isolation protocol, use the supernatant as the sample.

    Follow point 1. of the protocol.

Feces (sample stored in conservation solution StoolSave™ DNA Protection kit)

Additional reagents you will need:

For stool samples stored in the **StoolSave™ DNA Protection kit (cat # 006-10)**:

- **Bead-beat tubes** (2 ml screwed Bead-beat tubes containing beads mix), cat # K-PKCM-50
- **L3P** precipitation solution (100 µl per sample), cat # K-L3P-60

For stool samples stored in another preservation solution:

- **Bead-beat tubes** (2 ml screwed Bead-beat tubes containing beads mix), cat # K-PKCM-50
- **L3P** precipitation solution (100 µl per sample), cat # K-L3P-60
- **LSDE** buffer (additional 250 µl per sample),cat # K-LSDE-500
- **antifoam** (10 µl per sample), cat# K-AYS-1

1. **Feces stored in conservation solution StoolSave™ DNA Protection kit**:

   Transfer **500 µl** of stool sample suspended in conservation/ transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads.
   Add **500 µl** of **LSDE** buffer.

   Feces stored in another preservation solution:

   Before starting the process, mix the **LSDE** buffer with **antifoam**. Prepare the **LSDE-antifoam** mix by combining **750 µl** of **LSDE** buffer with **10 µl** of **antifoam** per sample. Prepare a volume sufficient for the number of isolated samples with a 10% excess. Mix well before use.

   Transfer **250 µl** of stool sample suspended in conservation/ transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads.
   Add **750 µl** of **LSDE-antifoam** buffer.

2. **Option A:** Bead Beating. Use the suitable tube bead beater with the following programme:
   3 x run of 20 sec at **maximum force** with **1 min** cool down.

   Option B: Place the tubes in the incubator with mixing function (e.g. Thermomixer) and vortex at **2000 RPM** for **30 min** at **room temperature**.

3. Centrifuge for **5 min** at **10 000 RPM**.

4. Transfer **500 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

5. Add **20 µl Proteinase K.**

6. Vortex the sample for **10 s** and incubate for **15 min** at **50 °C** with shaking **1400 RPM**.

   Optional RNA removal. Add **10 µl** of **RNAse** (cat # 1006-10) and incubate for **10 min** at **37 °C** with shaking at **1400 RPM.**

7. Add **100 µl** of **L3P** precipitation solution. Close the tube and mix whole content by inverting the tube.

8. Place on ice for **3 min.**

9. Centrifuge the sample for **5 min** at **10 000 RPM**.

10. **Attention.** In the isolation protocol, use the supernatant as the sample.

    Follow point 1. of the protocol.

Protocol

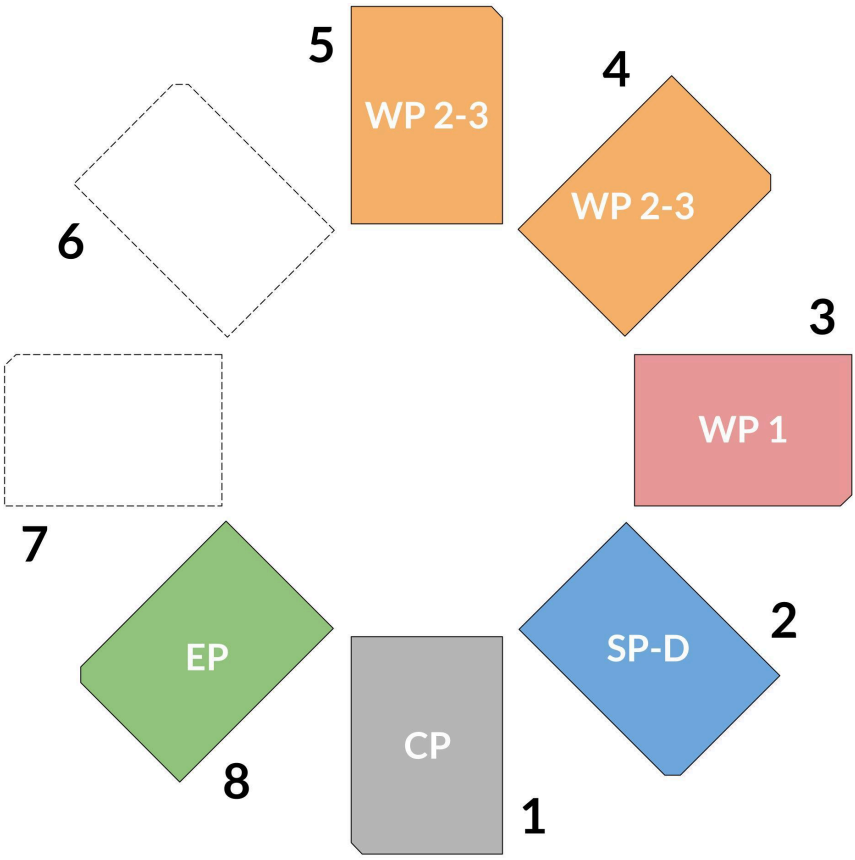
Protocol files

device	protocol name	protocol file	installation
Auto-Pure 96	MQ-UND-96	aabiot.com/protocols/magnifiq/96/MQ-UND-96.txt	1. Create folder "items" on a USB drive and copy the protocol file to it. 2. Insert the USB drive into a USB slot in the device. 3. On a device screen, go to Settings > Im.&Export > Import. 4. Select the protocol and tap "Import."

Extraction protocol

Before the isolation procedure, all plates should be centrifuged for **1 min** at **1000 RPM**. Centrifuge to remove remaining solution from the top of the protective film.

1. Remove the foil from the **SP-D** plate.
2. Add **400 µl** of samples to the wells of the **SP-D** plate.
3. Place the **tip comb 96** into the **CP** plate.
4. Remove the adhesive foil from the rest of the plates. Place the plates on the working table of the extraction device according to the scheme:



5 Run the protocol on your device.

6. After the program is over, remove the **EP** plate from the extraction device and seal it with protective film.

Note. For longer storage of extracted material, transfer it from the plate to appropriate tubes and store at 4 °C.

7. Discard remaining plates except the **CP** plate, which can be reused.

Additional information

Preparation of material in 1.5 ml Eppendorf tubes

Lysis of the material in 1.5 ml Eppendorf tubes should be carried out according to the respective procedure for 96 deep-well plate in the Material Preparation section. The following changes should be made:

- Incubation parameters
  Lower the incubation temperature by **5 °C** and shorten the time by **10 min**.
- Centrifugation parameters
  Centrifuge the tube for **2 min** at **10 000 RPM.**

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

LTE 2X

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

WP 1 plate, WP 2-3 plate

H225 Highly flammable liquid and vapor.
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P261 Avoid breathing dust/fume/gas/mist/vapours/ spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P301+P312+P330 If swallowed: Call a poison center/doctor/ if you feel unwell.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 If eye irritation persists: Get medical advice/ attention.

DANGER

SP-D plate

H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye damage.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P301+P312+P330 If swallowed: Call a poison center/doctor/ if you feel unwell.
P303+P361+P353 If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P304+P340 If inhaled: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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