A&A BIOTECHNOLOGY
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Manual

Genomic Mini Universal

Increased efficiency kit for genomic DNA purification from various types of biological materials.

catalog #	size
116U-50	50 isolations
116U-250	250 isolations

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

- One universal kit for DNA isolation from various types of material.
- Precise instructions for material preparation.
- Time-saving procedure.
- High quality of isolated DNA.

Sample type

sample type	sample size
Bacteria G-, G+ (cultures)	up to 1 ml
Yeast (cultures)	up to 1 ml
Cell cultures	up to 1 x 10^6
Blood: fresh or frozen	up to 200 µl
Semen	200 µl
Feces, environmental samples (soil, activated sediment, compost)	20 - 50 mg
Feces, environmental samples (soil, activated sediment, compost) stored in conservation solution	200 - 300 µl
Solid tissue	up to 20 mg
Dry swab	1 pc

Specification

protocol time	~ 15 min
elution volume	100 µl
elution solution	Tris buffer
binding capacity	20 µg DNA
downstream applications	qPCR, RT-qPCR, sequencing

Contents

	116U-50		116U-250		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
minicolumns	50 pcs	K-K01-50	250 pcs	K-K01-250	15–25 °C
2 ml tubes	100 pcs	K-PGR-100	500 pcs	K-PGR-500	15–25 °C
RA activation solution	22 ml	K-RA-22	110 ml	K-RA-110	15–25 °C
BL lysis buffer	15 ml	K-BL-15	70 ml	K-BL-70	15–25 °C
LSDE buffer	12 ml	K-LSDE-12	55 ml	K-LSDE-55	15–25 °C
RW binding solution	10 ml	K-RW-10	42 ml	K-RW-42	15–25 °C
W10 wash solution	28 ml	K-W10-28	140 ml	K-W10-140	15–25 °C
W11 wash solution	50 ml	K-W11-50	250 ml	K-W11-250	15–25 °C
Tris elution buffer (10 mM, pH 8,5)	30 ml	K-TRIS-30	125 ml	K-TRIS-125	15–25 °C
Proteinase K	1.1 ml	K-PRK-11A	5 x 1.1 ml	K-PRK-11A	2-8 °C*

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

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- incubator or thermoblock
- vortex
- microcentrifuge

Optional

- RNase (10 µl per sample), cat # 1006-10

Column preparation

Before starting the isolation procedure, it is important to activate the columns.

1. Add **400 µl** of **RA** activation solution directly onto the minicolumn.
2. Incubate for **5 min** at **room temp**.
3. Centrifuge for **1 min** at **10 000-15 000 RPM**.
4. Discard the filtrates.
5. Place the minicolumns into **the same** tubes.

Material preparation

Bacteria G-, G+ (cultures)

Additional reagents you will need:

Bacteria lysis kit (cat. # 604BK-50, 604BK-100)

- **BacBreaker** bacteria lysis enzyme mix (20 µl per sample)
- **BS** suspension buffer (200 µl per sample)

1. Transfer up to **1 ml** of bacterial culture sample to the 1.5 ml Eppendorf tube (not included). Centrifuge for **3 min** at **10 000 RPM**. Discard the supernatant.
2. Suspend the bacterial pellet in **200 µl** of **BS** buffer.
3. Add **20 µl BacBreaker** enzyme mix.

   Optional RNA removal. Add **10 µl** of **RNase** (cat # 1006-10).
4. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.
5. Add **200 µl** of **BL** lysis buffer and **20 µl Proteinase K**.
6. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.

7. Add **150 µl** of **RW** binding buffer.

8. Vortex the samples intensively for **10 s.**

9. Follow point 1. of the isolation protocol.

Yeast (cultures)

Additional reagents you will need:

Yeast lysis kit (cat. # 604YK-50, 604YK-100)

- **Lyticase (**20 µl per sample)
- **DTT RTU, ultrapure water** (10 µl 1M solution per sample)
- **BS** suspension buffer (200 µl per sample)

Prepare **1M DTT** solution. Add 1 ml of ultrapure water to a vial containing DTT powder to obtain 1M DTT solution. Mix or vortex until complete dissolution of DTT powder. Store solution at -20 °C.

1. Transfer up to **1 ml** of yeast culture to 1.5 ml Eppendorf tube (not included).
Centrifuge for **3 min** at **10 000 RPM**. Discard the supernatant.

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

3. Add **20 µl lyticase** and **10 µl 1M DTT.**

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

4. Vortex the sample for **10 s** and incubate for **20 min** at **37 °C**.

5. Add **200 µl** of **BL** lysis buffer and **20 µl Proteinase K**.

6. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.

7. Add **150 µl** of **RW** binding buffer.

8. Vortex the samples intensively for **10 s.**

9. Follow point 1. of the isolation protocol.

Cell cultures

1. Transfer the cell culture sample containing **1 x 10^6** cells to 1.5 ml Eppendorf tube (not included). Centrifuge for **2 min** at **10 000 RPM**. Discard the supernatant.
2. Suspend the cell pellet in **200 µl** of **Tris** buffer.

   Optional RNA removal. Add **10 µl** of **RNase** (cat # 1006-10).
3. Add **200 µl** of **BL** lysis buffer and **20 µl Proteinase K.**
4. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C** with shaking.
5. Add **150 µl** of **RW** binding buffer.
6. Vortex the samples intensively for **10 s.**
7. Follow point 1. of the isolation protocol.

Blood: fresh or frozen

1. Transfer **200 µl** of the sample to 1.5 ml Eppendorf tube (not included).

   Note. For blood volume less than 200 µl, add Tris buffer to a total volume of 200 µl.
2. Add **200 µl** of **BL** lysis buffer and **20 µl Proteinase K.**
3. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.
4. Add **150 µl** of **RW** binding buffer.
5. Vortex the sample for **10 s** and centrifuge for **20 s** at **10 000 RPM.**

   Note. Centrifuge to remove remaining material from lids of the tubes and deposit non-lysed material at the bottom of the tube.
6. Follow point 1. of the isolation protocol.

Semen

Additional reagents you will need:

- **DTT RTU** (10 µl 1M solution per sample), cat # 2010-10P

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 solution at -20 °C.

1. Transfer **200 µl** of the sample to 1.5 ml Eppendorf tube (not included).

   Note. For semen volume less than 200 µl, add Tris buffer to a total volume of 200 µl.

2. Add **20 µl Proteinase K** and **20 µl 1M DTT.**

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

3. Add **200 µl** of **BL** lysis buffer.

4. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.

5. Add **150 µl** of **RW** binding buffer.

6. Vortex the samples intensively for **10 s.**

7. Follow point 1. of the isolation protocol.

Feces, environmental samples (soil, activated sediment, compost)

Additional reagents you will need:

Microbiome lysis kit (cat. # 604MK-50, 604MK-100)

- **Bead-beat tubes** (2 ml screwed Bead-beat tubes containing beads mix)
- **L3P** precipitation solution (75 µl per sample)
- **LSDE** buffer (600 µl per sample)
- **antifoam** (10 µl per sample)

Before starting the process, mix the **LSDE** buffer with **antifoam**. Prepare the **LSDE-antifoam** mix by combining **600 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.

1. Transfer **20-50 mg** of sample to the 2 ml screwed Bead-beat tube containing beads mix.
   Add **600 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 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 **300 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

5. Add **20 µl** of **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 **75 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. Transfer **200 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

11. Add **200 µl** of **BL** lysis buffer and vortex the sample for **10 s.**

12. Add **150 µl** of **RW** binding buffer and vortex the sample for **10 s.**

13. Follow point 1. of the isolation protocol.

Feces, environmental samples (soil, activated sediment, compost) stored in conservation solution

Additional reagents you will need:

For 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 (75 µl per sample), cat # K-L3P-60

For samples stored in another preservation solution:

Microbiome lysis kit (cat. # 604MK-50, 604MK-100)

- **Bead-beat tubes** (2 ml screwed Bead-beat tubes containing beads mix)
- **L3P** precipitation solution (75 µl per sample)
- **LSDE** buffer (400 µl per sample)
- **antifoam** (10 µl per sample)

1. **Samples stored in conservation solution StoolSave™ DNA Protection kit**:
   Transfer **300 µl** of sample suspended in conservation/ transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads.
   Add **300 µl** of **LSDE** buffer.

   Samples stored in another preservation solution:
   Before starting the process, mix the **LSDE** buffer with **antifoam**. Prepare the **LSDE-antifoam** mix by combining **400 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 **200 µl** of sample suspended in conservation/ transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads.
   Add **400 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 **300 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

5. Add **20 µl** of **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 **75 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. Transfer **200 µl** of supernatant to a new 1.5 ml Eppendorf tube (not included).

11. Add **200 µl** of **BL** lysis buffer and vortex the sample for **10 s.**

12. Add **150 µl** of **RW** binding buffer and vortex the sample for **10 s.**

13. Follow point 1. of the isolation protocol.

Solid tissue

1. Transfer **up to 20 mg** of fragmented solid tissue to 1.5 ml Eppendorf tube (not included).

   Note. The tissue should be fragmented by cutting into pieces or spread in liquid nitrogen.

2. Add **200 µl** of **LSDE** buffer and **20 µl Proteinase K.**

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

3. Vortex the sample for **10 s** and incubate for **about 2 hours** until complete lysis at **50 °C**.

   Note. For maximum isolation efficiency, mix the samples by inverting the tubes a few times.

4. Add **200 µl** of **BL** lysis buffer.

5. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.

6. Centrifuge the sample for **2 min** at **15000 RPM**.
   Transfer the supernatant to a new 1.5 ml Eppendorf tube (not included).

7. Add **150 µl** of **RW** binding buffer.

8. Vortex the samples intensively for **10 s.**

9. Follow point 1. of the isolation protocol.

Dry swabs

1. Break or cut off part of the swab with the collected sample and place it in a 1.5 ml Eppendorf tube (not included).

   Note. The portion of the swab with the collected sample should fit completely into the tube.

2. Add **250 µl** of **Tris** buffer, **20 µl Proteinase K** and **250 µl** of **BL** lysis buffer.

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

3. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C**.
   Transfer the lysate to a new 1.5 ml Eppendorf tube (not included).

4. Add **150 µl** of **RW** binding buffer.

5. Vortex the samples intensively for **10 s.**

6. Follow point 1. of the isolation protocol.

Isolation protocol

1.	Apply samples onto the activated minicolumns.
2.	Centrifuge for **1 min** at **10 000 RPM**.
3.	Remove the minicolumns from the tubes. Discard the filtrates. Transfer the minicolumns to **new** 2 ml tubes (included).
4.	Add **500 µl** of **W10** wash solution.
5.	Centrifuge for **1 min** at **10 000 RPM**.
6.	Remove the minicolumns from the tubes. Discard the filtrates. Transfer the minicolumns to **new** 2 ml tubes (included).
7.	Add **500 µl** of **W11** wash solution. Mix by inverting the tubes a few times. **Note**. Mixing is intended to remove residual wash buffer from the inner walls of the column.
8.	Centrifuge for **1 min** at **10 000 RPM**.
9.	Remove the minicolumns from the tubes. Discard the filtrates. Dry the rims of the tubes from any leftover wash solution. Turn the tube upside down and gently touch it against a paper towel. Transfer the minicolumns into t**he same** tubes.
10.	Add **400 µl** of **W11** wash solution.
11.	Centrifuge for **1 min** at **15 000 RPM**.
12.	Transfer the minicolumns to **new** 1.5 ml tubes (not included).
13.	Add **100 µl** of **Tris** buffer at the bottom of the minicolumns.
14.	Incubate for **2 min** at **room temp.**
15.	Centrifuge for **1 min** at **10 000 RPM**.

16. Remove the minicolumns and store the tubes with purified DNA at **4 °C or -20 °C** until further use.

Additional information

The final DNA eluate may contain trace amounts of particles from the column membrane. The particles do not affect the quality of the isolated DNA. However, they may be of significance for spectrophotometric readings (A 230/260). Before spectrophotometric analysis, it is recommended to centrifuge the eluate for 1 min at maximum speed and take a sample from the top layer of the centrifuged solution.

Safety information

Proteinase K

DANGER

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.

RW binding buffer

DANGER

H225 Highly flammable liquid and vapor.
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 vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

W10 wash solution

DANGER

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, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

W11 wash solution

DANGER

H225 Highly flammable liquid and vapor.
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 vapors.
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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