

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

Specification

sample type	sample size
<u>Bacteria G-, G+ (cultures)</u>	up to 1 ml
<u>Yeast (cultures)</u>	up to 1 ml
Cell cultures	up to 1 x 10 ⁶
Blood: fresh or frozen	up to 200 µl
<u>Semen</u>	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
<u>Dry swab</u>	1 pc

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		11	6U-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 ℃
RA activation solution	22 ml	K-RA-22	110 ml	K-RA-110	15-25 ℃
BL lysis buffer	15 ml	K-BL-15	70 ml	K-BL-70	15-25 ℃
LSDE buffer	12 ml	K-LSDE-12	55 ml	K-LSDE-55	15-25 ℃
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)	20 ml	K-TRIS-20	100 ml	K-TRIS-100	15-25 ℃
Proteinase K	1.1 ml	K-PRK-11A	5 x 1.1 ml	K-PRK-11A	2-8 ℃*

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

Additional equipment and reagents

Necessary

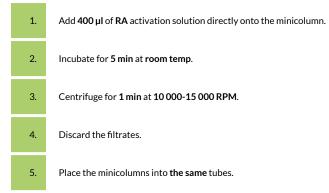
- 1.5 ml sterile Eppendorf tubes
- incubator or thermoblock
- vortex
- microcentrifuge

Optional

• RNase (10 µl per sample), <u>cat # 1006-10</u>

Column preparation

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

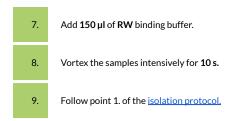


Material preparation

Bacteria G-, G+ (cultures)

Additional reagents you will need: Bacteria lysis kit (cat. # 604BK-50, 604BK-100)

٠	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 (<u>cat # 1006-10</u>).
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 .



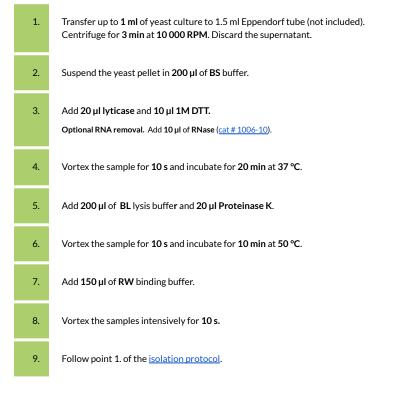
Yeast (cultures)

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)

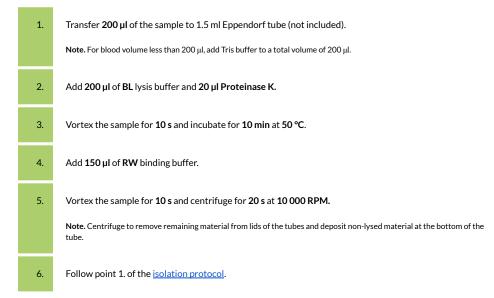
Prepare **1M DTT** solution. Add **1** ml of ultrapure water to a vial containing DTT powder to obtain **1M DTT** solution. Mix or vortex until complete dissolution of DTT powder. Store solution at -20 °C.



Cell cultures

1. Transfer the cell culture sample containing 1 x 10⁶ 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. Follow point 1. of the isolation protocol. 7.

Blood: fresh or frozen



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 $200\mu l$ of the sample to 1.5 ml Eppendorf tube (not included).
	Note. For semen volume less than 200 $\mu l,$ add Tris buffer to a total volume of 200 $\mu l.$
2.	Add 20 µl Proteinase K and 20 µl 1M DTT.
	Optional RNA removal. Add 10 μl of RNase (<u>cat # 1006-10</u>).
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 μ l 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 $300 \mu l$ 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.
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 .
6. 7.	
	Optional RNA removal. Add 10 μl of RNase (<u>cat # 1006-10</u>) and incubate for 10 min at 37 °C with shaking at 1400 RPM.
7.	Optional RNA removal. Add 10 μl of RNase (<u>cat # 1006-10</u>) and incubate for 10 min at 37 °C with shaking at 1400 RPM. Add 75 μl of L3P precipitation solution. Close the tube and mix whole content by inverting the tube.
7.	Optional RNA removal. Add 10 µl of RNase (<u>cat # 1006-10</u>) and incubate for 10 min at 37 °C with shaking at 1400 RPM. Add 75 µl of L3P precipitation solution. Close the tube and mix whole content by inverting the tube. Place on ice for 3 min .

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. <u>Samples stored in conservation solution StoolSave[™] DNA Protection kit</u>:

Transfer **300** μ I of sample suspended in conservation/transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads. Add **300** μ I 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** μ I of sample suspended in conservation/transportation solution to the 2 ml screwed Bead-beat tube containing zirconia beads. Add **400** μ I of LSDE-antifoam buffer.

2. <u>Option A:</u> 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.

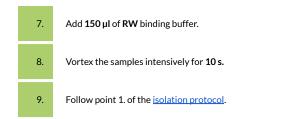
- 3. Centrifuge for **5 min** at **10 000 RPM**.
- 4. Transfer 300 µl of supernatant to a new 1.5 ml Eppendorf tube (not included).

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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 µ l 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 μI 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 <u>isolation protocol</u> .

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.
	Note. The dissue should be haginented by cutting into pieces of spread in inquid fitt ogen.
2.	Add 200 µl of LSDE buffer and 20 µl Proteinase K.
	Optional RNA removal. Add 10 µl of RNase (<u>cat # 1006-10</u>).
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).



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 <u>isolation protocol</u> .

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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