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Genomic DNA Isolation Kit

Code MBK0069
50 extractions

Intended use

Genomic DNA Isolation Kit is designed for the rapid preparation of genomic DNA from various tissue samples, cultured cells, bodily fluids and nasal or throat swabs.
Purification is based on spin column chromatography using a resin as the separation matrix. Resin specifically binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested and is completely compatible with PCR and Southern Blot analysis.
A specific procedure was optimized for DNA extraction from feather samples (Annex A) to be used in combination with FAST Avian Sexing PCR kit (code MBK0102).

Product description

The Genomic DNA Isolation Kit allows for the isolation of genomic DNA from various types of animal tissues or cell samples. In addition, a protocol is also provided for the purification of viral DNA. In all cases the genomic DNA is preferentially purified from other cellular proteinaceous components. Typical yields of genomic DNA will vary depending on the sample being processed.
Preparation time for a single sample is approximately 60 minutes, and each kit contains sufficient materials for 50 preparations.

Kit contents

Component	Product # MBK0069 (50 samples)
Digestion Buffer A	25 mL
Buffer SK	30 mL
Wash Solution A	18 mL
Elution Buffer B	30 mL
Proteinase K	12 mg
Spin Columns	50 pieces
Collection Tubes	50 pieces
Elution Tubes	50 pieces

Shipping conditions

Shipping at room temperature has no detrimental effect on the performance of this kit.

Storage

The Proteinase K should be stored at -20°C upon arrival and after reconstitution. All other solutions should be kept tightly sealed and stored at room temperature.

Precautions

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. **Buffer SK** contains guanidine hydrochloride and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment:

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 55 °C water bath or heating block

- 96–100% ethanol
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer
- RNase A (optional)
- PCR Grade Water
- Dithiothreitol 1M (only for DNA extraction from feather samples)

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary g-force.

Section 1. Preparation of Lysate from Various Cell Types

Notes Prior to Use:

• The steps for preparing the lysate are different depending on the starting material (**Step 1**). However, the subsequent steps are the same in all cases (**Steps 2-4**).
• Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
• A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
• Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
• Reconstitute the **Proteinase K** in 0.6mL of molecular biology grade water, aliquot in 120 µL fractions and store the unused portions at -20°C until needed.
• Add 42 mL of 96-100% ethanol to all the provided bottles of **Wash Solution A**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
• Preheat a water bath or heating block to 55°C.

1A. Lysate Preparation from Animal Tissues

Notes Prior to Use:

• Fresh or frozen tissues may be used for the procedure. Tissues should be immediately frozen and stored at -20°C or -70°C. Tissues may be stored at -70°C for several months.
• It is recommended that no more than 20mg of tissue be used, in order to prevent clogging of the column.

a. Excise up to 20 mg of tissue sample. Either frozen or fresh tissue may be used. Place the sample in a nuclease-free microfuge tube.

> **Note:** The tissue sample may be homogenized into a fine powder in liquid nitrogen to improve lysis efficiency.

b. Add 300 µL of **Digestion Buffer A** to the tissue sample. Homogenize the sample using tools such as a rotor -stator homogenizer or a microfuge-size pestle.

Optional RNase A Treatment:
If RNA - free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step 1c.

c. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

> **Note**: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of tissue being lysed. Lysis is considered

complete when a relatively clear lysate is obtained.

d. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing.

e. Add 300 µL of 96–100% ethanol. Mix by vortexing.

f. Proceed to Step 2: Binding to Column.

1B. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use:

- Cells grown in suspension or monolayer may be used.
- The maximum recommended input of cells is 3 x 10^6. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen cell pellets should not be subjected to repeated cycles of freeze and thaw prior to beginning the protocol.

1B(i). Cell Lysate Preparation from Cells Growing in a Monolayer

a. Detach cells by standard trypsinization method or cell scraper. Transfer an appropriate amount to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.
b. Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.
c. Add 200 µL of **Digestion Buffer A** to the cell pellet. Mix by gentle vortexing.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step 1B(i)d.

d. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

> **Note:** Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

e. Add 200 µL of **Buffer SK** to the lysate. Mix by vortexing.

f. Add 200 µL of 96–100% ethanol. Mix by vortexing.

g. Proceed to Step 2: Binding to Column.

1B(ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a. Transfer an appropriate amount of cells to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.

b. Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.

c. Add 200µL of **Digestion Buffer A** to the cell pellet. Mix by gentle vortexing.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step 1B(ii) d.

d. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

> **Note:** Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is

considered complete when a relatively clear lysate is obtained.

e. Add 200 µL of **Buffer SK** to the lysate. Mix by vortexing.

f. Add 200 µL of 96–100% ethanol. Mix by vortexing.

g. Proceed to Step 2: Binding to Column.

1C. Lysate Preparation from Bodily Fluids or Swabs

Notes Prior to Use:

- Up to 150 µL of bodily fluids including blood and saliva can be processed.
- Fresh samples of bodily fluids are recommended. Frozen samples may be used, however the yield of genomic DNA may be decreased.

a. For Bodily Fluid: Transfer up to 150 µL of a bodily fluid sample to a 1.5 mL microfuge tube (not provided). Adjust the volume to 300µL by adding **Digestion Buffer A**. Proceed to Step 1Cc.

b. For Swabs: Using sterile techniques, cut the cotton tip where the cells were collected and place into 300 µL of **Digestion Buffer A** in a microcentrifuge tube. Vortex gently and incubate for 5 minutes at room temperature. Remove the cotton tip with sterile forceps.

Alternatively, transfer the cells (in **Digestion Buffer A**) to a 1.5 mL microcentrifuge tube (not provided). Proceed to Step 1Cc.

c. Optional RNase A treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with step 1Cd.

d. Add 12 µL of Proteinase K to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

> **Note:** Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

e. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing.

f. Add 300 µL of 96–100% ethanol. Mix by vortexing.

g. Proceed to Step 2: Binding to Column.

1D. Lysate Preparation for Viral DNA

Notes Prior to Use:

- For the isolation of integrated viral DNA, follow Section 1A if the starting material is animal tissue, follow Section 1B if the starting material is cell culture, and follow Section 1C if the starting material is bodily fluid such as blood.
- For the isolation of DNA from free viral particles, follow the protocol provided below.
- Up to 150 µL of viral suspension or bodily fluids can be processed.
- Fresh samples are recommended. Frozen samples may be used, however the yield of genomic DNA may be decreased.

a. Transfer up to 150 µL of a sample to a 1.5 mL microfuge tube (not provided). Adjust the volume to 300µL by adding **Digestion Buffer A**.

Optional RNase A treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well.

b. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and incubate at 55°C for 1 hour.

Note: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

c. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing.

d. Add 300 µL of 96–100% ethanol. Mix by vortexing.

e. Proceed to Step 2: Binding to Column.

Section 2. Genomic DNA Purification from all types of lysate

The remaining steps of the procedure for the purification of genomic DNA are the same from this point forward for all the different types of lysate.

2. Binding to Column

a. Assemble a spin column with a provided collection tube. Apply up to 600 µL of the mixture to the spin column assembly. Cap the column and centrifuge the unit for 3 minutes at 5,200 x g (~ 8,000 RPM).

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin at 14,000 x g (~14,000 RPM) for 2 minutes.

b. After centrifugation, discard the flowthrough, and reassemble the spin column with its collection tube.

c. Repeat Step 2a and Step 2b until all the lysate mixture has passed through the column.

3. Washing Bound DNA

a. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).

b. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.

c. Apply 500 µL of **Wash Solution A** to the column, and centrifuge for 2 minutes at 14,000 x g (~14,000 RPM).

d. Carefully detach the spin column from the collection tube and discard the collection tube and flowthrough.

Note: If any liquid is left on the side of the spin column, discard the flowthrough and reassemble the spin column with its collection tube. It is highly recommended to spin for an additional 1 minute at 14,000 x g (~14,000 RPM) in order to completely dry the column.

4. Elution of Clean DNA

a. Assemble the spin column (with DNA bound to the column) with a provided 1.7 mL **Elution tube.**

b. Add 200 µL of **Elution Buffer B** to the center of the resin bed. Centrifuge for 1 minute at 3,000 *x g* (~6,000 RPM). A portion of the **Elution Buffer B** will pass through the column which allows for hydration of the DNA to occur.

c. Centrifuge at 14,000 *x g* (~14,000 RPM) for an additional 2 minutes to collect the total elution volume.

d. (Optional): An additional elution may be performed if desired. Another 200 µL of **Elution Buffer B** may be added to the column and centrifuged at 3,000 x g for 1 minute into a new elution tube. Then, centrifuge the column at 14,000 x g for an additional 2 minutes. The yield can be improved by an additional 20-30% when this second elution is performed.

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Annex A
Procedure for the extraction of genomic DNA from feather samples

Notes Prior to Use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Reconstitute the **Proteinase K** in 0.6 mL of molecular biology grade water, aliquot in 120 µL fractions and store the unused portions at -20°C until needed.
- Add 42 mL of 96-100% ethanol to all the provided bottles of **Wash Solution A**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat a water bath or heating block to 55°C.
- Prepare a fresh solution of Dithiothreitol 1M (reagent not included in the kit).

1. Lysate preparation

a) Put the feather in a 1.5 ml tube. If the feather is longer than 2 cm, cut its terminal portion and put it in a 1.5 ml tube.
b) Add to each sample 300 µl of **Digestion Buffer A** and 18 µl of **Dithiothreitol 1M** (final concentration: 60 mM)
c) Add 12 µl of **Proteinase K** to the suspension. Mix well by vortexing and incubate at 55°C for 1 h. Mix by vortexing every 30 minutes during incubation.

***IMPORTANT NOTE**: Ensure that the terminal portion of the feather is completely immersed in the buffer during the incubation.*

d) Centrifuge for 3 minutes at 12,000 x g (~12,000 RPM). After centrifugation, transfer the suspension in a new 1.5 ml tube.
e) Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing.
f) Add 300 µL of 96–100% ethanol. Mix by vortexing.

2. Binding to Column

g) Assemble a spin column with a provided collection tube. Apply up to 600 µL of the mixture to the spin column assembly. Cap the column and centrifuge the unit for 3 minutes at 5,200 x g (~ 8,000 RPM).

***Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin at 14,000 x g (~14,000 RPM) for 2 minutes.*

h) After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
i) Repeat step *g* and step *h* until all the lysate mixture has passed through the column.

3. Washing Bound DNA

j) Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
k) After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
l) Apply 500 µL of **Wash Solution A** to the column and centrifuge for 2 minutes at 14,000 x g (~14,000 RPM).
m) Carefully detach the spin column from the collection tube and discard the collection tube and flowthrough.

***Note:** if any liquid is left on the side of the spin column, discard the flowthrough and reassemble the spin column with its collection tube. It is highly recommended to spin for an additional 1 minute at 14,000 x g (~14,000 RPM) in order to completely dry the column.*

4. Elution of Clean DNA

n) Assemble the spin column (with DNA bound to the column) with a provided 1.7 mL **Elution tube.**
o) Add 100 µL of **Elution Buffer B** to the center of the resin bed. Centrifuge for 1 minute at 3,000 *x g* (~6,000 RPM). A portion of the **Elution Buffer B** will pass through the column which allows for hydration of the DNA to occur.
p) Centrifuge at 14,000 *x g* (~14,000 RPM) for an additional 2 minutes to collect the total elution volume.

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.