Protocol for bacteria

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to the section “Preparation of working solutions” in the IFU of the DBG-Spin™ blood DNA kit.
- Set an incubator or water bath to 56 °C.

1. Prepare sample

Up to 1 mL of bacterial culture can be used for the preparation depending on, for example, density of culture, culture medium, and bacterial strain.

Centrifuge up to **1 mL culture** for **5 min** at **8,000 × g**. Remove supernatant.

2. Pre-lyse sample

Resuspend the pellet in **180 µL DBG-TL™ Lysis Buffer** by pipetting up and down. Add 25 µL Proteinase K. Vortex vigorously and incubate at 56 °C until complete lysis is obtained (at least 1–3 h). Vortex occasionally during incubation or use a shaking incubator.

Samples can be incubated overnight as well.

If RNA-free DNA is crucial for downstream applications, an RNase digest may be performed: Add 20 µL RNase A (20 mg/mL) solution (not included) and incubate for an additional 5 min at room temperature.

***Hard-to-lyse bacteria:** Some strains, especially Gram-positive bacteria, are more difficult to lyse. In such cases, a preincubation with a lytic enzyme is necessary: Resuspend the pelleted cells in 20 mM Tris/HCl; 2 mM EDTA; 1 % Triton X-100; pH 8 (instead of DBG-TL™ Lysis Buffer) supplemented with a final concentration of 20 mg/mL lysozyme or 0.2 mg/mL lysostaphin and incubate for 30 – 60 min at 37 °C. Add 25 µL Proteinase K, incubate at 56 °C until complete lysis is obtained.*

Proceed with step 3 of the standard protocol (see section 1).

Standard protocol for human or animal tissue and cultured cells

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to the section “Preparation of working solutions” in the IFU of the DBG-Spin™ blood DNA kit.
- Set an incubator or water bath to 56 °C.
- Pre-heat Wash Buffer BE to 70 °C.

1. Prepare sample

Tissue

Cut 25 mg human or animal tissue into small pieces.

Place the sample in a microcentrifuge tube (not provided).

Proceed with step 2.

Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer (for example Polytron®, Ultra-Turrax®): Add 25 mg of tissue to a 1.5 mL microcentrifuge tube (not provided), add 50–75 µL phosphate buffered saline (PBS) and homogenize.

Cultured cells

Resuspend up to **10^7 cells** in a final volume of **200 µL DBG-TL™ Lysis Buffer**. Add **25 µL Proteinase K** solution and **200 µL Buffer B3**. Vortex to mix and incubate the sample at 70 °C for 10–15 min. **Proceed with step 4.**

2. Pre-lyse sample

Add **180 µL DBG-TL™** Lysis Buffer and **25 µL Proteinase K** solution. Vortex to mix. Be sure that the samples are completely covered with lysis solution.

+180 µL T1
+25 µL
Proteinase K
Mix

If processing several samples, Proteinase K and Buffer DBG-TL™ Lysis Buffer may be premixed directly before use. Do not mix DBG-TL™ Lysis Buffer and Proteinase K more than 10–15 min before addition to the sample: Proteinase K tends to self-digest in DBG-TL™ Lysis Buffer without substrate.

Incubate at **56 °C** until complete lysis is obtained **(at least 1–3 h)**. Vortex occasionally during incubation or use a shaking incubator.

56 °C,
1–3 h
or
56°C,
overnight

Samples can be incubated overnight as well. If RNA-free DNA is crucial for downstream applications, a RNase digest may be performed: Add 20 µL RNase A (10 mg/mL) solution (not included) and incubate for an additional 5 min at room temperature.

3. Lyse sample

Vortex the samples. Add **200 µL Buffer B3**, vortex vigorously and incubate at **70 °C** for **10 min**. Vortex briefly.

If insoluble particles are visible, centrifuge for 5 min at high speed (e.g. 11,000 x g) and transfer the supernatant to a new microcentrifuge tube (not provided).

+ 200 µL B3
70 °C,
10 min

4. Adjust DNA binding conditions

Add **210 µL ethanol (96–100 %)** to the sample and vortex vigorously.

After addition of ethanol a stringy precipitate may become visible. This will not affect the DNA isolation. Be sure to load all of the precipitate on the column in the following step.

+ 210 µL
ethanol
Vortex

5. Bind DNA

For each sample, place one **DBG-Spin™ Blood DNA Kit Column** into a Collection Tube. Apply the sample to the column. Centrifuge for **1 min** at **11,000 × g**. Discard Collection Tube with flowthrough and place the column in a new Collection Tube (provided).

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

Load samples

11,000 × *g*,
1 min

6. Wash silica membrane

1 st wash

Add **500 µL Buffer BW**. Centrifuge for **1 min** at **11,000 × g**. Discard flowthrough and place the column back into the Collection Tube.

+500 µL BW
11,000 × *g*,
1 min

2nd wash

Add **600 µL Buffer B5** to the column and centrifuge for **1 min** at **11,000 × g**. Discard flowthrough and place the column back into the Collection Tube.

+600 µL B5
11,000 × *g*,
1 min

7. Dry silica membrane

Centrifuge the column for **1 min** at **11,000 × g**.
Residual ethanol is removed during this step.

11,000 × *g*,
1 min

8. Elute highly pure DNA

Place the DBG-Spin™ Blood DNA Kit Column into a 1.5mL microcentrifuge tube (not provided) and add **100 µL Buffer BE (70 °C).**
Incubate at room temperature for 1min.
Centrifuge **1 min** at **11,000 × g**.

+100 µL BE

RT
1 min
11,000 × *g*,
1 min

For alternative elution procedures see below.
In addition to the standard method (recovery rate about 70–90 %), several modifications are possible to increase yield, concentration, and convenience. Use elution buffer for one of the following procedures:

•High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid can be eluted.
•High concentration: Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be approximately 30 % higher than with standard elution. The yield of eluted nucleic acid will be about 80 %.
•High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–100 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.
•Elution at 70 °C: For certain sample types, heating the elution buffer to 70 °C increases the DNA yield.
Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. Note: Elution Buffer BE (5 mM Tris/HCl, pH 8.5) provided with the kit does not contain EDTA.
For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied elution buffer and storage, especially long term, at -20 °C. Freeze-thaw cycles will have no effect on most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g., > 10 kbp). Multiple freeze-thaw cycles or storing DNA at 4 °C or room temperature may influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.