

## Protocol for purification of genomic DNA from insects

### Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to 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

Homogenize **not more than 50 mg insects** under liquid nitrogen and transfer the powder into a 1.5 mL microcentrifuge tube (not provided).

Proceed with step 2 of the standard protocol with addition of Proteinase K.

## 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<sup>7</sup> 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.

*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.

*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.*



+180 µL T1  
+25 µL  
Proteinase K  
Mix



56 °C,  
1–3 h  
or  
56 °C,  
overnight

### 3. Lyse sample

Vortex the samples. Add **200  $\mu$ L 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  $\times$  g) and transfer the supernatant to a new microcentrifuge tube (not provided).*



+ 200  $\mu$ L B3  
70 °C,  
10 min

### 4. Adjust DNA binding conditions

Add **210  $\mu$ L 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  $\mu$ L  
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  $\times$  g**. Discard Collection Tube with flowthrough and place the column in a new Collection Tube (provided).



Load samples



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

11,000  $\times$  g,  
1 min

### 6. Wash silica membrane

#### 1 st wash

Add **500  $\mu$ L Buffer BW**. Centrifuge for **1 min** at **11,000  $\times$  g**. Discard flowthrough and place the column back into the Collection Tube.



+500  $\mu$ L BW  
11,000  $\times$  g,  
1 min

#### 2nd wash

Add **600  $\mu$ L Buffer B5** to the column and centrifuge for **1 min** at **11,000  $\times$  g**. Discard flowthrough and place the column back into the Collection Tube.



+600  $\mu$ L B5  
11,000  $\times$  g,  
1 min

### 7. Dry silica membrane

Centrifuge the column for **1 min** at **11,000  $\times$  g**.

Residual ethanol is removed during this step.



11,000  $\times$  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  $\mu$ L Buffer BE (70 °C)**. Incubate at room temperature for 1min.



+100  $\mu$ L BE

Centrifuge **1 min** at **11,000  $\times$  g**.



RT  
1 min  
11,000  $\times$  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.*