

# BILATEST DNA Tissue Kit



100 Extractions

## Product description

The BILATEST DNA Tissue Kit is designed for the simple and fast DNA Purification from Tissues (10 mg) and 2 - 4 mm Mouse Tail Sections.

The complete protocol takes approximately 30 minutes, the expected yield is:

2 - 4 mm (10 mg) mouse tail: 15 - 25 µg DNA

10 mg liver: 24 - 40 µg DNA

## Included reagents

Reagent	Volume
(1) Magnetic Beads	7.8 ml
(2) Lysis Buffer	11 ml
(3) Binding Buffer	28 ml
(4) Washing Buffer A	55 ml
(5) Washing Buffer B	55 ml
(6) Washing Buffer C	110 ml
(7) Elution Buffer	11 ml

The **Elution Buffer (7)** included in this kit is 10 mM Tris-HCl pH 8.0. TE buffer pH 8.0 can also be used without any protocol adjustments. Water pH 8.0 may also be used, in this case we recommend an elution time of 10 - 15 minutes at 55° C to ensure the highest yield of purified DNA.

This kit contains enough materials for 100 isolations from 10 mg tissue (e.g. 2 - 4 mm Mouse Tail Sections).

## Required Materials

Proteinase K stock solution (10 mg/ml in H<sub>2</sub>O)

This kit is optimized for use with BILATEC Magnetic Separators (e.g. BILATEST magnetic separator M 12+12 for 1.5ml tubes, Order-No. 210141).

## Storage Conditions

This kit may be stored at room temperature (15 - 25°C) and is stable for at least 1 year following delivery.

## Samples and Protocol Adjustments

### ! Important Note !

Before use, **Proteinase K** has to be added to **Lysis Buffer (2)** to a final concentration of 250 µg/ml (e.g. add 25 µl Proteinase K stock solution (10 mg/ml) to 1 ml **Lysis Buffer (2)**).

## UV Measurements

In some cases the manual user may find some traces of the magnetic beads left in the eluate after removal from the tube. Such particles will not interfere with PCR and most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional application of the magnet to the eluate for 2 minutes in order to separate any traces of particles. For pure DNA the expected  $A_{260}/A_{280}$  ratio is between 1.7 - 2.0. The  $A_{260}$  value should be between 0.1 and 1.0 for accurate readings.

## Purification Protocol for 10 mg Tissue

1. Cut up to 10 mg tissue into small pieces and place it in **100 µl Lysis Buffer (2)** (containing **Proteinase K**). Incubate with agitation (600 rpm) at **56°C until lysis is complete**. Occasional vortexing will decrease incubation time. Lysis overnight is possible and does not influence the preparation. After lysis, spin down material that is not lysed (e.g. bones, hairs) and use the supernatant for the next steps. (Working with the not lysed material will not affect the quality of the DNA, but will make the isolation process more difficult).
2. Premix **75 µl** resuspended **Magnetic Beads (1)** with **263 µl Binding Buffer (3)** and add the mixture to the lysate. Mix with 10 pipetting strokes and incubate **10 minutes at room temperature**.
3. Following incubation, place the tube in a Magnetic Separator to draw the **Magnetic Bead/DNA Complex** to the side of the tube. Leave 2 minutes, then discard the supernatant and remove the tube from the magnet position.
4. Add **500 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the wash buffer by pipetting the bead pellet up and down 15 times.
5. Separate the **Magnetic Bead/DNA Complex** in the magnetic separator for 1 minute. Aspirate and discard supernatant.
6. Remove tube from the magnet position and repeat the washing procedure (steps 4 & 5) using **Washing Buffer B (5)**.
7. After removing the last traces of **Washing Buffer B (5)**, leave tube in the Magnetic Separator.
8. With the tube in the Magnetic Separator and the beads attracted to the side of the tube gently add **1 ml** (or as large a volume as possible) **Washing Buffer C (6)**, being careful not to disrupt the pellet. Leave **90 seconds** without resuspending the bead pellet and then carefully remove and discard the supernatant.  
(**Note:** resuspension of the bead pellet in **Washing Buffer C (6)** may reduce the final DNA yield.)
9. Add **100 µl** (or another suitable volume) of **Elution Buffer (7)** to the tube and thoroughly resuspend the **Magnetic Bead/DNA Complex** by pipetting the pellet up and down 10 to 15 times.
10. Incubate the suspension for **10 minutes at 55°C** with agitation (1000 rpm) to facilitate complete DNA elution.
11. Following DNA elution place the tube in the Magnetic Separator for 2 minutes or until all the **Magnetic Beads (1)** have separated from the eluate. Transfer the eluate containing the purified DNA to a clean tube. (For UV measurements it is recommended to put the tube containing the eluate again in the magnetic separator and leave for 2 minutes).

### Purification Protocol for dried blood spots

1. Cut 3 mm diameter punches from a dried blood spot. Place 3 – 4 punched-out circles in a micro-centrifuge tube, add **180 µl Lysis Buffer (2) (without Proteinase K)** and incubate at **85°C**.
2. Centrifuge the tube briefly, add **20 µl Proteinase K** and incubate **1 hour at 56°C**.
3. Add **75 µl** resuspended **Magnetic Beads (1)**, premixed with **263 µl Binding Buffer (3)**, to the lysate. Mix with 10 pipetting strokes and incubate 10 minutes at room temperature.
4. Following incubation, place the tube in a Magnetic Separator to draw the **Magnetic Bead/DNA Complex** to the side of the tube. Leave 2 minutes, then discard supernatant and remove the tube from the magnet position.
5. Add **500 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the washing buffer by pipetting the bead pellet up and down 15 times.
6. Separate the **Magnetic Bead/DNA Complex** in the magnetic separator for 1 minute. Aspirate and discard supernatant
7. Remove tube from the magnet position and repeat the washing procedure (steps 4 & 5) using **Washing Buffer B (5)**.
8. After removing the last traces of **Washing Buffer B (5)**, leave tube in the Magnetic Separator.
9. With the tube in the Magnetic Separator, and the beads attracted to the side of the tube, gently add **1 ml** (or as large a volume as possible) **Washing Buffer C (6)**, being careful not to disrupt the pellet. Leave **90 seconds without resuspending** the bead pellet and then carefully remove and discard the supernatant.  
  
(**Note:** resuspension of the bead pellet in **Washing Buffer C (6)** may reduce the final DNA yield.)
10. Add **100 µl** (or another suitable volume) of **Elution Buffer (7)** to the tube and thoroughly resuspend the **Magnetic Bead/DNA Complex** by pipetting the pellet up and down 10 to 15 times.
11. Incubate the suspension for **10 minutes at 55°C**, with agitation (1000 rpm) to facilitate complete DNA elution.
12. Following DNA elution place the tube in the Magnetic Separator for 2 minutes or until all the **Magnetic Beads (1)** have separated from the eluate. Transfer the **eluate** containing the purified DNA to a clean tube. (For UV measurements it is recommended to put the tube containing the eluate again in the magnetic separator and leave for 2 minutes).