BILATEST
Genomic DNA Kit 10

100 Extractions (Blood)

Product description

The BILATEST Genomic DNA Kit 10 is designed for the simple and fast isolation of genomic DNA from 10 µl whole blood.
The complete protocol takes approximately 15 minutes, the expected yield from 10µl whole blood from healthy persons is about 0.2 – 0.4 µg DNA.

Included reagents

Reagent	Volume
(1) Lysis Buffer	7 ml
(2) Magnetic Beads	0.82 ml
(3) Binding Buffer	11 ml
(4) Washing Buffer A	20 ml
(5) Washing Buffer B	31 ml
(6) Elution Buffer	10 ml

The **Elution buffer (6)** included in this kit is 10 mM Tris-HCl, pH 8.0. TE buffer, pH 8.0 may also be used without any protocol adjustments. If the Elution Buffer is replaced with water (pH 8.0), we recommend an elution time of 10 - 15 minutes at 55 °C to maximize the DNA yield.

Required Materials

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 and Safety Information

Stored at room temperature (15-25°C) this kit is stable for at least 12 months. The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling.

Samples and Protocol Adjustments

The included protocol is suitable for fresh, non-coagulated, and frozen blood. Using this method, 0.5 - 2 % of the eluate is normally sufficient as template for PCR amplification. The protocol is scaleable for volumes greater than 10 µl blood.
This kit is optimized for DNA purification from normal healthy blood samples. In some cases, where an above normal amount of white cells is present, increasing the amount of magnetic beads may increase the final yield. Due to the high DNA content in buffy coat samples, we recommend to use only half the volume of starting material with the separation protocol. For dried blood samples, after addition of the **Lysis Buffer (1)**, we recommend a one-hour incubation at 50°C with shaking, in order to facilitate the separation of the DNA from other sample components.
For further technical information see section "Troubleshooting" or contact technical support at BILATEC.

Purification Protocol from up to 10 µl of Blood (detailed protocol)

1. Place **10 µl blood** sample in a tube. Add **65 µl Lysis Buffer (1)**, mix well with 6-8 pipetting strokes and incubate **1 minute** at room temperature.

2. Add **8 µl** resuspended **Magnetic Beads (2)** and **110 µl Binding Buffer (3)** to the tube. Mix with 6-8 pipetting strokes and incubate **5 minutes** at room temperature.
   !!Ensure that the Magnetic Beads (2) are in suspension before dispensing; if they have been standing for a long period of time it may help to vortex them briefly before removal**!!**
   Magnetic Beads (2) and **Binding Buffer (3)** can be added separately or premixed for multiple samples.

3. Following incubation, place the tube in a magnetic separator to draw the **Magnetic bead/DNA complex** to the side of the tube. Leave **1 minute**, then discard supernatant and remove the tube from the magnet position.

4. Add **190 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the washing buffer by aspirating the beads 6-8 times.

5. Separate the **Magnetic Bead/ DNA complex** in the magnetic separator, discard supernatant. Pipette off any remaining traces of **Washing Buffer A (4)**.

6. With the tube in the magnetic separator (the beads attracted to the side of the tube), gently add **300 µl Washing Buffer B (5)**, being careful not to disrupt the pellet. Leave **30 seconds without resuspending the pellet** and then carefully remove and discard the supernatant.
   (**Note:** a longer incubation time or resuspension of the bead pellet in **Washing Buffer B (5)** may reduce the final DNA yield.)

7. Add **50 µl** (or another suitable volume) of **Elution Buffer (6)** to the tube and thoroughly resuspend the **Magnetic Bead/DNA complex** by mixing the pellet with 10 to 15 pipetting strokes. Lower elution volumes may be used to increase the final DNA concentration.

8. Incubate the suspension for **5-10 minutes at 55°C** (occasional agitation may facilitate the complete DNA elution).

9. Following DNA elution place the tube in the magnetic separator for 1 minute or until all the beads have separated from the eluate. Transfer the **eluate** containing the purified DNA into a clean tube.

10. Store the DNA under appropriate conditions or use a small amount as template for PCR.

BILATEC AG, Heidelbergerstr. 5-7, D-68519 Viernheim
Tel. +49-6204-70 15 97-0, Fax +49-6204-70 15 97-9
Email: sales@bilatec.com, Internet: www.bilatec.com

Purification Protocol from up to 10 µl of Blood (quick protocol)

1. Mix 1 to 10 µl whole blood and **65 µl Lysis Buffer (1)** in a microfuge tube.

2. Incubate **1 minute** at room temperature.

3. Add **8 µl** resuspended **Magnetic Beads (2)** and **110 µl Binding Buffer (3)** to the tube and mix.

4. Separate **Magnetic Bead/DNA Complex**, discard supernatant then remove tube from the magnetic separator.

5. Add **190 µl Washing Buffer A (4)** to the tube. Resuspend the pellet with 6-8 pipetting strokes.

6. Separate **Magnetic Bead/DNA Complex**, discard supernatant and **leave tube in the magnetic separator**!

7. Remove all remaining traces of **Washing Buffer A (4)**.

8. Gently add **300 µl Washing Buffer B (5)**. Leave 30 seconds **without resuspending the bead pellet** and then carefully remove and discard the supernatant.

9. Add **50 µl** (or another suitable volume) **Elution Buffer (6)** and resuspend **Magnetic Bead/DNA Complex**.

10. Incubate 5 minutes at 55°C, with occasional agitation.

11. Separate the **Magnetic Beads (2)** for approximately 1 minute.

12. Transfer the eluate to a clean tube.

Troubleshooting

Problem	Possible Cause	Recommendation/Solution
Low yield	Sample condition	-Yield is dependent on the leukocyte concentration in the starting sample. The **BILATEST®** DNA kits are optimized for use with normal healthy blood samples. -When an extraordinarily high amount of DNA is present, one can decrease the volume of sample or increase the amount of Magnetic Beads used. -We recommend to cut the sample volume in half when processing buffy coat samples.
	Washing Buffer A (4) not removed sufficiently	-**Washing buffer A (4)** contains ethanol which can inhibit elution if not removed sufficiently. -Ensure that as much buffer as possible is removed from the tube before proceeding with **Washing Buffer B (5)**.
	Incomplete Elution	-Verify that the elution temperature was correct and, if necessary, extend the elution time by an additional five minutes
	Insufficient Lysis or Binding to Magnetic Beads	-Mix samples thoroughly upon addition of lysis and binding buffer. -In some cases it may help to lengthen the lysis time.
	Bead pellet not properly resuspended in elution step	-Resuspend bead pellet in elution buffer until the pellet is homogeneously dispersed.
	Water used in elution step	-Water can be used in place of the included Elution Buffer, however, the elution time should be doubled to achieve a comparable yield.
	Bead pellet resuspended or incubated for extended period in **Wash Buffer B (5)**	-Do not resuspend bead pellet in **Washing Buffer B (5)** -Do not incubate bead pellet for more than 1 minute in the presence of **Washing Buffer B (5)**.
	RNA contamination	-Add 10 µl RNase A (20 µg/µl) per 100 µl eluate and incubate 10 minutes at room temperature. Repeat purification protocol omitting the lysis buffer step.
A_{260}/A_{280} ratio is too high	Protein contamination	-Beads not sufficiently resuspended during washing steps. -If necessary, repeat purification protocol omitting the lysis buffer step.
A_{260}/A_{280} ratio is too low	Residual beads in eluate	-Incomplete separation of the Magnetic Beads from the eluate can increase the background of UV measurements. -Repeat magnetic separation and transfer eluate to a clean tube. -Residual Magnetic Beads will not affect most downstream processes.
Precipitate in reagent bottle	Bottles stored below room temperature.	-Warm reagent bottle in water bath to redissolve precipitate.
	Old Sample, or sample has been repeatedly frozen and thawed	-To reduce DNAse activity in frozen blood samples, thaw them quickly in a 37 °C water bath and then place on ice until use.
Degraded DNA	DNAse contamination	-Verify DNAse contamination of buffers. -Replace elution buffer with fresh TE or 10 mM Tris-HCl, pH 8.0 if necessary.
	Water used in elution step	-Check that the water pH is above 7.0 to avoid acid hydrolysis of the eluate over time.

BILATEC AG, Heidelbergerstr. 5-7, D-68519 Viernheim
Tel. +49-6204-70 15 97-0, Fax +49-6204-70 15 97-9
Email: sales@bilatec.com, Internet: www.bilatec.com