

BILATEST Genomic DNA Kit 100



100 Extractions (Blood)

Product description

The BILATEST Genomic DNA Kit 100 is designed for the simple and fast isolation of genomic DNA from 100µl whole blood.

The complete protocol takes approximately 25 minutes, the expected yield from 100µl whole blood from normal healthy whole blood is 2 - 4 µg DNA.

Included reagents

Reagent	Volume
(1) Lysis Buffer	13ml
(2) Magnetic Beads	1.45ml
(3) Binding Buffer	38ml
(4) Washing Buffer A	62ml
(5) Washing Buffer B	62ml
(6) Washing Buffer C	102ml
(7) Elution Buffer	11ml

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.

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), the kit is stable for at least 12 months following delivery.

The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling.

Samples and Protocol Adjustments

The included protocol is sufficient for most blood samples: fresh, non-coagulated, and frozen. This kit is optimized for DNA purification from normal healthy human blood samples. Using blood from animals or with very high cell concentrations we recommend increasing the volume of **Lysis Buffer (1)**, up to a maximum of three times the blood volume.

Correspondingly, the volume of **Binding Buffer (3)** has to be adjusted so that the concentration of this Buffer in the total mixture (**Blood + Lysis Buffer (1) + Magnetic Beads (2) + Binding Buffer (3)**) is 60%. In some cases, where an above normal amount of white cells are present, increasing the amount of **Magnetic Beads** may increase the final yield.

Using this method 0.5 – 2% of the eluate is normally sufficient template for PCR. The protocol is also scaleable up to 1 ml whole blood.

UV Measurements

In some cases, traces of magnetic beads will remain in the eluate after removal from the tube. These beads 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 magnetic field to the eluate for 3 minutes in order to remove any traces of magnetic beads. 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 100 µl of Blood

1. Place **100 µl blood** sample in a tube. Add **125 µl Lysis Buffer (1)**, mix well (8 to 10 pipetting strokes), and incubate **5 minutes** at room temperature.
2. Add **14 µl** resuspended **Magnetic Beads (2)**, premixed with **360 µl DNA Binding Buffer (3)**. Mix with 10 pipetting strokes and incubate **5 minutes** at room temperature.
3. 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.
(**Note:** the magnetic beads will not be visible in this step. Therefore, it is important to remove the supernatant from the opposite side of the magnets, in order not to remove magnetic beads.)
4. Add **600 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the washing buffer by aspirating the bead pellet 15 times.
5. Separate the **Magnetic bead / DNA complex** in the magnetic separator, discard supernatant and remove tube from the magnet position.
6. Repeat the washing procedure (steps 4 and 5) using **Washing Buffer B (5)**.
7. After completely removing the last traces of **Washing buffer B (5)**, **leave the tube in the magnetic separator**.
8. With the tube in the magnetic separator (the beads attracted to the side of the tube), gently add **1 ml Washing Buffer C (6)**, being careful not to disrupt the pellet. Leave for **90 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 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 mixing the pellet with 10 to 15 pipetting strokes.
10. Incubate the suspension for **5 to 10 minutes at 55°C** (occasional agitation may facilitate complete DNA elution).
11. Following DNA elution place the tube in the magnetic separator for **2 minutes** or until all the beads 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).

Quick Protocol (please read detailed protocol before proceeding)

1. Mix **100 µl blood** and **125 µl Lysis Buffer (1)** in a tube.
Incubate **5 minutes** at room temperature.
2. Add **14 µl** resuspended **Magnetic Beads (2)**, premixed with **360 µl Binding Buffer (3)**, and mix well.
Incubate **5 minutes** at room temperature.
3. Separate **Magnetic Bead / DNA complex** for **2 minutes**, discard supernatant then remove tube from the magnetic separator.
4. Thoroughly resuspend bead pellet in **600 µl Washing Buffer A (4)**.
5. Separate **Magnetic Bead / DNA complex** for **1 minute** and discard supernatant.
6. Remove tube from the magnetic separator and repeat the washing procedure (steps 4 and 5) using **Washing Buffer B (5)**.
7. **Leave tube in the magnetic separator.**
8. With the beads attracted to the magnet, gently add **1 ml Washing Buffer C (6)**. Leave 90 seconds **without resuspending the bead pellet**. Carefully remove and discard the supernatant.
9. Add **100 µl** (or another suitable volume) **Elution Buffer (7)** and resuspend **Magnetic Bead / DNA complex**.
10. Incubate 5 – 10 minutes at 55°C, with occasional agitation.
11. Separate the **Magnetic Beads** and 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 ² 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 (2) used. -We recommend that one reduces the sample volume in half when processing buffy coat samples.
	Wash Buffer B (5) not removed sufficiently	- Wash Buffer B (5) 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 Wash Buffer C (6) .
	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 C (6)	-Do not resuspend bead pellet in Wash Buffer C (6) -Do not incubate bead pellet for more than 1 minute in the presence of Wash Buffer C (6) .
A₂₆₀ /A₂₈₀ ratio is too high	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.
A₂₆₀ /A₂₈₀ ratio is too low	Protein contamination	-Beads not sufficiently resuspended during washing steps. -If necessary, repeat purification protocol omitting the lysis buffer step.
	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.
Degraded DNA	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.
	DNase contamination	-Verify DNase contamination of buffers. -Replace elution buffer with fresh TE or 10 mM Tris-Cl, 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.