



chemagic DNA Blood100 Kit

DNA purification from 100 µl blood

for general purposes

Kit Components

Magnetic Beads	1.4 ml
Lysis Buffer 1	12.5 ml
Binding Buffer 2	36 ml
Wash Buffer 3	60 ml
Wash Buffer 4	60 ml
Wash Buffer 5	100 ml
Elution Buffer 6	10 ml

The **Elution Buffer** is 10 mM Tris-HCl pH 8.0; one can also use TE buffer pH 8.0.

This kit contains enough materials for 100 isolations from 100 µl of blood and is optimized for use with **chemagic** Stand 2x12 (Magnetic Separator).

Completion time: Approximately 25 minutes.

Expected yield from normal healthy whole blood: 2 - 4 µg DNA.

Required Materials

- **chemagic** Stand 2x12 (Art. No. 300)

Storage Conditions and Safety Information

This kit may be stored at room temperature (15 – 25 °C) and is stable for at least 1 year following delivery. The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling.



Samples and Protocol Adjustments

The **Elution Buffer 6** 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.

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 2** has to be adjusted so that the concentration of **Binding Buffer 2** in the total mixture (Blood + **Lysis Buffer 1** + **Magnetic Beads** + **Binding Buffer 2**) 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 amplification. The protocol is also scaleable up to 1 ml whole blood.

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 100 µl of Blood

1. Place the 100 µl **blood** sample and 125 µl **Lysis Buffer 1** in a micro-centrifuge tube, mix well (8 to 10 pipetting strokes) and then incubate **5 minutes** at room temperature.
2. Add 14 µl resuspended **Magnetic Beads**, premixed with 360 µl **Binding Buffer 2**, to the tube. Mix with 10 pipetting strokes and incubate **5 minutes** at room temperature.
3. Following incubation, place the tube in a **chemagic** Stand 2x12 (magnet position) 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 supernatant is dark and the magnetic beads will not be visible. Therefore, it is important to pipette off the supernatant from the opposite side of the tube from the Magnetic force, so as not to inadvertently aspirate magnetic beads.)

4. Add 600 µl **Wash Buffer 3** 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 a **chemagic** Stand 2x12 (magnet position) for **1 minute**. Aspirate and discard supernatant.
6. **Remove tube from the magnet position** and repeat the washing procedure (steps 4 and 5) using **Wash Buffer 4**.
7. After removing the last traces of **Wash Buffer 4**, **leave tube in the magnet position**.
8. With the tube in the magnet position, and the beads attracted to the side of the tube, gently add 1 ml (or as large a volume as possible) **Wash Buffer 5**, being careful not to disrupt the pellet. Leave **90 seconds without resuspending the bead pellet** and then carefully remove and discard the supernatant.



Resuspension of the bead pellet in Wash Buffer 5 may reduce the final DNA yield.

9. Add 100 µl (or another suitable volume) of **Elution Buffer 6** 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 5 to 10 minutes at 55 °C, with occasional agitation to facilitate complete DNA elution.
11. Following DNA elution place the tube in a **chemagic** Stand 2x12 (magnet position) for 2 minutes or until all the **Magnetic 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 μ l **blood** and 125 μ l **Lysis Buffer 1** in micro-centrifuge tube.
Incubate **5 minutes** at room temperature.
2. Add 14 μ l resuspended **Magnetic Beads**, premixed with 360 μ l **Binding Buffer 2**, 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 **chemagic** Stand 2x12 (magnet position).
4. Thoroughly resuspend bead pellet in 600 μ l **Wash Buffer 3**.
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 **Wash Buffer 4**.
7. **Leave tube in the magnet position.**
8. With the beads attracted to the magnet, gently add 1 ml **Wash Buffer 5**. Leave 90 seconds **without resuspending the bead pellet**. Carefully remove and discard the supernatant.
9. Add 100 μ l (or another suitable volume) **Elution Buffer 6** 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	<ul style="list-style-type: none"> Yield is dependent on the leukocyte concentration in the starting sample. The chemagen 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 that one halve the sample volume when processing buffy coat samples.
	Wash Buffer 4 not removed sufficiently	<ul style="list-style-type: none"> Wash Buffer 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 Wash Buffer 5.
	Incomplete Elution	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Resuspend bead pellet in Elution Buffer 6 until the pellet is homogeneously dispersed.
	Water used in elution step	<ul style="list-style-type: none"> Water can be used in place of the included Elution Buffer 6, however, the elution time should be doubled to achieve a comparable yield.
	Bead pellet resuspended or incubated for extended period in Wash Buffer 5	<ul style="list-style-type: none"> Do not resuspend bead pellet in Wash Buffer 5 Do not incubate bead pellet for more than 1 minute in the presence of Wash Buffer 5.
A₂₆₀ /A₂₈₀ ratio is too high	RNA contamination	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Magnetic Beads not sufficiently resuspended during washing steps. If necessary, repeat purification protocol omitting the lysis buffer step.
	Residual beads in eluate	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> Warm reagent bottle in water bath to redissolve precipitate.
Degraded DNA	Old Sample, or sample has been repeatedly frozen and thawed	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Check that the water pH is above 7.0 to avoid acid hydrolysis of the eluate over time.