



chemagic DNA Blood10k Kit

DNA purification from 10 ml of whole blood

for general purposes

Kit Components

Magnetic Beads	55 ml
Lysis Buffer 1	475 ml
Binding Buffer 2	1500 ml
Wash Buffer 3	1500 ml
Wash Buffer 4	1500 ml
Wash Buffer 5	2000 ml
Elution Buffer 6	100 ml

This kit contains enough materials for 50 isolations from 10 ml of blood and is optimized for use with **chemagic** Stand 50k (see section “**The Magnetic Separator**”).

Completion time: Approximately 45 minutes.

Expected yield from normal healthy whole blood: 200 - 400 µg DNA.

Required Materials

chemagic Stand 50k Type A (Art. No. 305)

Storage Conditions 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 protocol can be used for sample volumes from 6 – 10 ml of whole blood. When the starting volume differs from the standard protocol (10 ml blood), an adjustment of the **Binding Buffer 2** volume has to be made, so that the concentration of **Binding Buffer 2** in the total mixture (Blood + **Lysis Buffer 1** + **Magnetic Beads** + **Binding Buffer 2**) is 60 %.

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 15 minutes at 55 °C to ensure a high 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 is present, increasing the amount of **Magnetic Beads** may increase the final yield.

UV Measurements

In some cases the manual user may find some traces of the magnetic beads left in the eluate after the final separation. 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 3 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 fall between 0.1 and 1.0 for accurate readings.

The Magnetic Separator

This kit is designed for use with the **chemagic** Stand 50k Type A (Art. No. 305), which has two different separation positions. The position with one magnet only is intended for the elution step. If another separator is used we recommend increasing the separation times in step 5 (depending on the strength of the magnet used). The magnetic separation in the following protocol steps should be long enough for all the beads to visibly adhere to the side of the tube.



Purification Protocol for 10 ml of Blood

1. Briefly vortex the blood sample and place 10 ml into an appropriate tube.
2. Add 9.5 ml of **Lysis Buffer 1** to the tube and mix by pipetting (5x with 25 ml pipette) or vortexing (for **30 seconds**), incubate for **5 minutes** at room temperature.
3. Pre-mix 1.1 ml of resuspended **Magnetic Beads** and 30 ml of **Binding Buffer 2** in a separate tube.
4. Following the Lysis step, add the **Magnetic Beads / Binding Buffer 2** mixture to the sample. Mix thoroughly by pipetting (8 x with 25 ml or 50 ml pipette) and then incubate **5 minutes**.
5. Following incubation, place the tube in a **chemagic** Stand 50k and leave **4 minutes** to separate all of the beads. Discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
6. Add 30 ml of **Wash Buffer 3** to the tube, mix by vortexing vigorously for **1 minute** and then incubate **2 minutes**.
7. Place the tube in the **chemagic** Stand 50k and leave **3 minutes** to separate all of the beads. Discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
6. Repeat the washing procedure (steps 6 and 7) using **Wash Buffer 4**. After removing all traces of **Wash Buffer 4**, **leave tube in the magnetic separator**.
8. While leaving the tube against the magnet, and the beads attracted to the side of the tube, gently add 40 ml of **Wash Buffer 5** being careful not to disturb the pellet. Immediately following a **1 minute** incubation, discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
9. Add 1 - 2 ml of **Elution Buffer 6** and then completely resuspend the **Magnetic Beads / DNA complex** pellet by vortexing vigorously for **20 seconds**. Incubate the suspension in a 55 °C water bath for **10 minutes**, with gentle agitation to facilitate DNA elution.
10. Place the tube in the **chemagic** Stand 50k and leave for **3 minutes** to separate all of the beads. Then carefully remove the eluate containing the purified DNA. (If beads are still present in the eluate repeat the magnetic separation step.)

Completion Time: Approximately 45 minutes

Expected Yield: 200 - 400 µg DNA