



chemagic DNA Blood5k Kit

DNA purification from 3 - 5 ml of whole blood

for general purposes

Kit Components

Magnetic Beads	33 ml
Lysis Buffer 1	330 ml
Binding Buffer 2	1050 ml
Wash Buffer 3	900 ml
Wash Buffer 4	900 ml
Wash Buffer 5	1800 ml
Elution Buffer 6	50 ml

This kit contains enough materials for 50 isolations from 3 - 5 ml of blood and is optimized for use with **chemagic** Stand 50k (see section “**The Magnetic Separator**”).

Completion time: Approximately 40 minutes.

Expected yield from normal healthy whole blood: 100 - 200 µg DNA from 5 ml blood.

Required Materials

- **chemagic** Stand 50k Type B (Art. No. 303)

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 **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 B (Art. No. 303), which has two separation positions. 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 3 ml of Blood

1. Briefly vortex the blood sample and place 3 ml into a 50 ml tube.
2. Add 3.5 ml **Lysis Buffer 1** to the tube and mix by pipetting (3 x with 10 ml pipette) or by vortexing (for 10 s), incubate **5 minutes** at room temperature.
3. Pre-mix 350 µl resuspended **Magnetic Beads** and 10.5 ml **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 (5 x the whole volume with 25 ml pipette) or by vigorous vortexing (for 10 s), then incubate **5 minutes**.
5. Following incubation, place the tube in a **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. Add 10 ml **Wash Buffer 3** to the tube and thoroughly resuspend the pellet by vigorous vortexing for **30 seconds**. Incubate for **2 minutes**. place the tube in a **chemagic** Stand 50k to draw all of the beads to the side of the tube (leave **2 minutes** to separate). Discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
6. Repeat the washing procedure (step 6) using **Wash Buffer 4**. After removing the last traces of **Wash Buffer 4**, leave tube in the **chemagic** Stand 50k.
8. While leaving the tube in the **chemagic** Stand 50k, and the beads attracted to the side of the tube, gently add 20 ml of **Wash Buffer 5** being careful not to disrupt the pellet. Immediately following a **1 minute** incubation, discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
9. Add 0.5-1 ml **Elution Buffer 6** and then completely resuspend the **Magnetic Beads / DNA complex** pellet by vortexing 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 a **chemagic** Stand 50k and leave **3 minutes** to attract all of the beads and 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 40 minutes

Expected Yield: 60 - 120 µg DNA

Version 050824

Further Questions?

Phone +49 (0) 2401 805-501

chemagen technical
support

Mail support@chemagen.de



Purification Protocol for 5 ml of Blood

1. Briefly vortex the blood sample and place 5 ml into a 50 ml tube.
2. Add 5.5 ml **Lysis Buffer 1** to the tube and mix by pipetting (5 x with 25 ml pipette) or by vortexing (for 10 s), incubate **5 minutes** at room temperature.
3. Pre-mix 550 µl resuspended **Magnetic Beads** and 17 ml **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 (5x the whole volume with 25ml pipette) or by vigorous vortexing (for **30 seconds**) 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 15 ml **Wash Buffer 3** to the tube and thoroughly resuspend the pellet by vigorous vortexing for **40 seconds**. Incubate for **2 minutes**. Place the tube in a **chemagic** Stand 50k to draw all of the beads to the side of the tube (leave **2 minutes** to separate). Discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
7. Repeat the washing procedure (step 6) using **Wash Buffer 4**. After removing the last traces of **Wash Buffer 4**, leave tube in the **chemagic** Stand 50k.
8. While leaving the tube in the **chemagic** Stand 50k, and the beads attracted to the side of the tube, gently add 30 ml of **Wash Buffer 5** being careful not to disrupt the pellet. Immediately following a **1 minute** incubation, discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
9. Add 1 ml **Elution Buffer 6** and then completely resuspend the **Magnetic Beads / DNA complex** pellet by vortexing 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 a **chemagic** Stand 50k and leave **3 minutes** to attract all of the beads and 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 40 - 45 minutes

Expected Yield: 100 - 200 µg DNA

Version 050824

Further Questions?

Phone +49 (0) 2401 805-501

chemagen technical
support

Mail support@chemagen.de