For Laboratory Use Only. Not Intended For Use in Diagnostic Procedures.

chemagic DNA Blood5k Kit

DNA purification from 5 ml of whole blood

Kit Components

Magnetic Beads
Lysis Buffer 1
Binding Buffer 2
Wash Buffer 3
Wash Buffer 4
Wash Buffer 5
Wash Buffer 6
Elution Buffer 7
Protease

This kit contains enough material for 100 isolations from 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 and Safety Information

This kit may be stored at room temperature (15 – 20 °C) and is stable for at least 1 year following delivery. The reconstituted **Protease** is stable for 2 months at 2 – 8 °C or at –20 °C.

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 3 – 5 ml of whole blood. The use of higher sample volumes than 5 ml can cause significant reduction in yield. When the starting volume differs from the

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standard protocol (5 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 7** 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.

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 traces of the **Magnetic Beads** are 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 inreasing the separation times in step 4 (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.

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Purification Protocol for 5 ml of Blood

1. Briefly vortex the blood sample and place 5 ml into an appropriate tube.
2. Add 20 µl **Protease** and then 7.5 ml of **Lysis Buffer 1** to the tube and mix thoroughly by pipetting (5x with 25 ml pipette) or vortexing (for **30 seconds**), incubate for **5 minutes** at room temperature.
3. Following the lysis step, add 600 µl of well resuspended **Magnetic Beads** and then 19 ml **Binding Buffer 2** to the sample. Mix thoroughly by pipetting (8x with 25 ml or 50 ml pipette) and then incubate **5 minutes**.
4. Following incubation, place the tube in the **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.
5. Add 10 ml of **Wash Buffer 3** to the tube, mix by vortexing vigorously for **1 minute** and then incubate **2 minutes**.
6. Place the tube in the **chemagic** Stand 50k and leave **3 minutes** to separate all beads. Discard the supernatant and then remove the tube from the **chemagic** Stand 50k.
7. Repeat the washing procedure (steps 5 and 6) using 10 ml of **Wash Buffer 4** and then using 10 ml of **Wash Buffer 5**. After removing all traces of **Wash Buffer 5**, **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 30 ml of **Wash Buffer 6** being careful not to disturb the pellet. Following a **1 minute** incubation, carefully discard the supernatant and then remove the tube from the **chemagic** Stand 50k (**longer incubation than 1 minute can reduce the yield**).
9. Add 0.5 - 1 ml of **Elution Buffer 7** and then completely resuspend the **Magnetic Beads** / **DNA complex** pellet by pipetting up and down or vortexing vigorously for **20 seconds**. Incubate the suspension for **10 minutes** at room temperature with gentle agitation to facilitate DNA elution. Optional: Incubate the suspension in a 55 °C water bath for **5 minutes**, with gentle agitation to facilitate DNA elution. In some cases this may increase the final yield.
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.)

Version 120313

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