



# chemagic DNA Blood10 Kit

*for general purposes*

## Kit Components

<b>Magnetic Beads</b>	0.8 ml
<b>Lysis Buffer 1</b>	6.5 ml
<b>Binding Buffer 2</b>	11 ml
<b>Wash Buffer 3</b>	19 ml
<b>Wash Buffer 4</b>	30 ml
<b>Elution Buffer 5</b>	10 ml

The **Elution Buffer 5** 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 15 minutes

**Expected Yield:** 0.2 - 0.4 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 10 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: coagulated, non-coagulated, and frozen. Using this method, 0.5 – 2 % of the eluate is normally sufficient template for PCR amplification. This protocol is also scaleable for sample volumes greater than 10 µl.

This kit is optimized for DNA purification from normal healthy blood samples. In cases where an above normal amount of white cells are present, increasing the amount of **Magnetic Beads** may increase the final yield.



## DNA Purification from up to 10 $\mu$ l of Blood (detailed protocol)

1. Pipette 1 to 10  $\mu$ l **whole blood** and 65  $\mu$ l **Lysis Buffer 1** in a microfuge tube. Mix well with 6 - 8 pipetting strokes and incubate 1 minute at room temperature.
2. Add 8  $\mu$ l **resuspended Magnetic Beads** and 110  $\mu$ l **Binding Buffer 2** to the tube. Mix well with 6 - 8 pipetting strokes and incubate **5 minutes** at room temperature. Ensure that the **Magnetic Beads** are in suspension before dispensing; if they have been standing for a long period of time it may help to vortex them briefly before removal. **Magnetic Beads** and **Binding Buffer 2** can be added separately or premixed for multiple samples.
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. Wait 30 seconds or until all the beads have been attracted to the magnet. Discard the supernatant and then **remove the tube from the magnet position**.
4. Add 190  $\mu$ l **Wash Buffer 3** to the tube and resuspend the pellet with 6 to 8 pipetting strokes.
5. Separate the **Magnetic Bead / DNA Complex** in a **chemagic** Stand 2x12 (magnet position) and discard the supernatant. Pipette off any remaining traces of **Wash Buffer 3**.
6. With the tube in the in the magnet position, and the beads attracted to the side of the tube, gently add 300  $\mu$ l (or as large a volume as possible) **Wash Buffer 4**, being careful not to disrupt the pellet. Leave **30 seconds** without resuspending the bead pellet and then carefully remove and discard the supernatant.



***A longer incubation time or resuspension of the bead pellet in Wash Buffer 4 may reduce the final DNA yield.***

7. Add 50  $\mu$ l (or another suitable volume) of **Elution Buffer 5** to the tube and thoroughly resuspend the **Magnetic Bead/DNA Complex** by pipetting the pellet up and down 10 to 15 times. Lower elution volumes may be used to increase the final DNA concentration.
8. Incubate the suspension for 5 minutes at 55 °C, with occasional agitation to facilitate complete DNA elution.
9. Following DNA elution place the tube in a **chemagic** Stand 2x12 (magnet position) for 1 minute or until all the **Magnetic Beads** have separated from the eluate. Transfer the **eluate** containing the purified DNA to a clean tube.
10. Store the DNA under appropriate conditions or use a small amount as template for PCR.



### **Quick Protocol** (please read detailed protocol before proceeding)

1. Mix 1 to 10  $\mu\text{l}$  **whole blood** and 65  $\mu\text{l}$  **Lysis Buffer 1** in a microfuge tube.
2. Incubate **1 minute** at room temperature.
3. Add 8  $\mu\text{l}$  **resuspended Magnetic Beads** and 110  $\mu\text{l}$  **Binding Buffer 2** to the tube and mix.
4. Separate **Magnetic Bead / DNA Complex**, discard supernatant then remove tube from the magnet position.
5. Add 190  $\mu\text{l}$  **Wash Buffer 3** to the tube. Resuspend the pellet with 6 to 8 pipetting strokes.
6. Separate **Magnetic Bead / DNA Complex**, discard supernatant and **leave tube in the chemagic Stand 2x12 (magnet position)!**
7. Remove all remaining traces of **Wash Buffer 3**.
8. Gently add 300  $\mu\text{l}$  **Wash Buffer 4**. Leave 30 seconds **without resuspending the bead pellet** and then carefully remove and discard the supernatant.
9. Add 50  $\mu\text{l}$  (or another suitable volume) **Elution Buffer 5** and resuspend **Magnetic Bead / DNA Complex**.
10. Incubate 5 minutes at 55 °C, with occasional agitation.
11. Separate the **Magnetic Beads** for approximately 1 minute.
12. Transfer the **eluate** to a clean tube.

**Completion Time: Approximately 15 minutes**

**Expected Yield: 0.2 - 0.4  $\mu\text{g}$  DNA**



## Troubleshooting

Problem	Possible Cause	Recommendation/Solution
<b>Low yield</b>	Sample condition	<ul style="list-style-type: none"> <li>Yield is dependent on the leukocyte concentration in the starting sample. The <b>chemagic</b> DNA Kits are optimized for use with normal healthy blood samples.</li> <li>When an extraordinarily high amount of DNA is present, one can decrease the volume of sample or increase the amount of Magnetic Beads used.</li> <li>We recommend that one reduce the sample volume in half when processing buffy coat samples.</li> </ul>
	<b>Wash Buffer 3</b> not removed sufficiently	<ul style="list-style-type: none"> <li><b>Wash buffer 3</b> contains ethanol which can inhibit elution if not removed sufficiently.</li> <li>Ensure that as much buffer as possible is removed from the tube before proceeding with <b>Wash Buffer 4</b>.</li> </ul>
	Incomplete Elution	<ul style="list-style-type: none"> <li>Verify that the elution temperature was correct and, if necessary, extend the elution time by an additional five minutes</li> </ul>
	Insufficient Lysis or Binding to Magnetic Beads	<ul style="list-style-type: none"> <li>Mix samples thoroughly upon addition of lysis and binding buffer.</li> <li>In some cases it may help to lengthen the lysis time.</li> </ul>
	Bead pellet not properly resuspended in elution step	<ul style="list-style-type: none"> <li>Resuspend bead pellet in elution buffer until the pellet is homogeneously dispersed.</li> </ul>
	Water used in elution step	<ul style="list-style-type: none"> <li>Water can be used in place of the included Elution Buffer, however, the elution time should be doubled to achieve a comparable yield.</li> </ul>
	Bead pellet resuspended or incubated for extended period in <b>Wash Buffer 4</b>	<ul style="list-style-type: none"> <li>Do not resuspend bead pellet in <b>Wash Buffer 4</b></li> <li>Do not incubate bead pellet for more than 1 minute in the presence of <b>Wash Buffer 4</b>.</li> </ul>
	RNA contamination	<ul style="list-style-type: none"> <li>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.</li> </ul>
<b>A<sub>260</sub>/A<sub>280</sub> ratio is too high</b>	Protein contamination	<ul style="list-style-type: none"> <li>Beads not sufficiently resuspended during washing steps.</li> <li>If necessary, repeat purification protocol omitting the lysis buffer step.</li> </ul>
<b>A<sub>260</sub>/A<sub>280</sub> ratio is too low</b>	Residual beads in eluate	<ul style="list-style-type: none"> <li>Incomplete separation of the <b>Magnetic Beads</b> from the eluate can increase the background of UV measurements.</li> <li>Repeat magnetic separation and transfer eluate to a clean tube.</li> <li>Residual <b>Magnetic Beads</b> will not affect most downstream processes.</li> </ul>
	Bottles stored below room temperature.	<ul style="list-style-type: none"> <li>Warm reagent bottle in water bath to redissolve precipitate.</li> </ul>
<b>Precipitate in reagent bottle</b>	Old Sample, or sample has been repeatedly frozen and thawed	<ul style="list-style-type: none"> <li>To reduce DNase activity in frozen blood samples, thaw them quickly in a 37 °C water bath and then place on ice until use.</li> </ul>
<b>Degraded DNA</b>	DNase contamination	<ul style="list-style-type: none"> <li>Verify DNase contamination of buffers.</li> <li>Replace elution buffer with fresh TE or 10 mM Tris-HCl, pH 8.0 if necessary.</li> </ul>
	Water used in elution step	<ul style="list-style-type: none"> <li>Check that the water pH is above 7.0 to avoid acid hydrolysis of the eluate over time.</li> </ul>