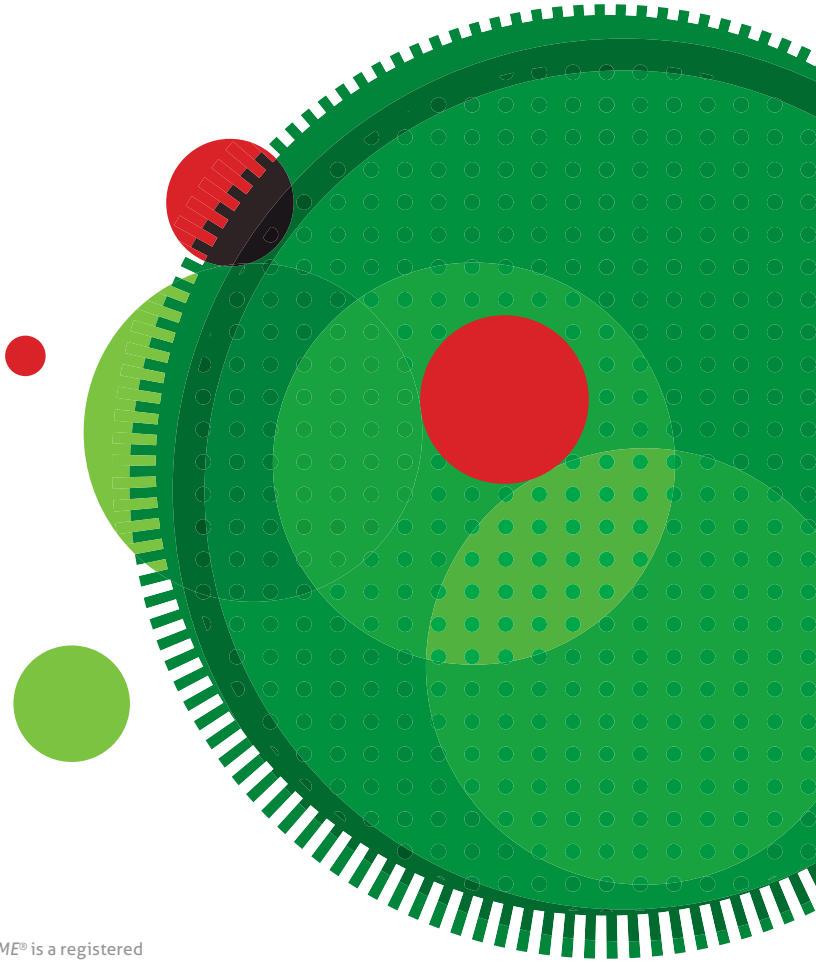
EXTRACTME®
DNA BLOOD KIT

Cat. No. EM05
Version: 1.2018

# Kit for DNA isolation from whole blood, plasma, serum, buffy coat, lymphocytes and body fluids



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## I. INTENDED USE

The ***EXTRACTME*** **DNA BLOOD KIT** is designed for a rapid and efficient purification of high quality (genomic, mitochondrial and viral) DNA from whole blood (fresh or frozen, human or mammalian), plasma, serum, buffy coat, lymphocytes and body fluids. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

## II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

| NUMBER OF ISOLATIONS | 10 ISOLATIONS | 50 ISOLATIONS | 250 ISOLATIONS | Storage Conditions [1] |
|---|---|---|---|---|
| **Catalogue number** | EM05-010 | EM05-050 | EM05-250 | |
| **RBC Lysis Buffer** (Red Blood Cell Lysis Buffer) | 10 ml | 50 ml | 250 ml | RT |
| **BL Buffer** (Lysis Buffer) | 3.8 ml | 19 ml | 94 ml | RT |
| ⚠ **Proteinase K** * (lyophilized) | 1 tube | 1 tube | 5 tubes | -20°C [2] |
| **Proteinase Buffer** | 200 µl | 320 µl | 1.6 ml | RT |
| **BB Buffer (conc.)** ** (Binding Buffer) | 1.8 ml | 10 ml | 44 ml | RT |
| **BW1 Buffer (conc.)** ** (Wash Buffer) | 3.3 ml | 17 ml | 82 ml | RT |
| **BW2 Buffer (conc.)** ** (Wash Buffer) | 1.4 ml | 9 ml | 33 ml | RT |
| **Elution Buffer** | 2 ml | 10 ml | 5 x 10 ml | RT |
| **DNA Purification Columns** | 10 pcs | 50 pcs | 5 x 50 pcs | RT |
| **Collection Tubes** (2 ml) | 10 pcs | 50 pcs | 5 x 50 pcs | RT |

[1] RT – room temperature (+15°C to +25°C)

[2] After reconstitution, **Proteinase K** should be stored **at -20°C**.

* Prior to the first use, add 320 µl **Proteinase Buffer** to a tube containing **Proteinase K** lyophilizate (in the kit for 10 isolations 200 µl of a buffer should be added).

** Before using for the first time, add the appropriate quantity of **96–100% ethanol** to **BB**, **BW1** and **BW2 Buffers** (see the instructions on the bottle label and in the table below). It is recommended to mark the bottle containing added alcohol.

| NUMBER OF ISOLATIONS | 10 ISOLATIONS | 50 ISOLATIONS | 250 ISOLATIONS |
|---|---|---|---|
| **Catalogue number** | EM05-010 | EM05-050 | EM05-250 |
| **BB Buffer** | 1.8 ml | 10 ml | 44 ml |
| **96–100% ethanol** | 2.7 ml | 15 ml | 66 ml |
| Total volume | 4.5 ml | 25 ml | 110 ml |
| **BW1 Buffer** | 3.3 ml | 17 ml | 85 ml |
| **96–100% ethanol** | 3.3 ml | 17 ml | 82 ml |
| Total volume | 6.6 ml | 34 ml | 164 ml |
| **BW2 Buffer** | 1.4 ml | 9 ml | 33 ml |
| **96–100% ethanol** | 3.3 ml | 21 ml | 77 ml |
| Total volume | 4.7 ml | 30 ml | 110 ml |

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

### Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

## III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 96–100% ethanol PFA
- sterile microcentrifuge tubes (1.5–2 ml)
- automatic pipettes and sterile DNase-free tips
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- dry block heater or water bath (up to 55°C)
- vortex mixer

## IV. PRINCIPLE

DNA purification procedure consists of five steps and utilizes spin minicolumns with membranes, which efficiently and selectively bind nucleic acids. In the first isolation step, red blood cells are lysed. The cells contain no DNA and are a potential source of PCR inhibitors. They must therefore be separated from the white blood cells prior to DNA isolation. Then the white blood cells are subjected to enzymatic lysis by Proteinase K in optimized BL Buffer. At this stage, all cell membranes and proteins are degraded. After the addition of chaotropic salts, lysate is applied to purification minicolumn membrane and DNA is bound. A two-step washing stage effectively removes impurities and enzyme inhibitors. Purified DNA is eluted using either a low ionic strength buffer (Elution Buffer) or water (pH 7.0–9.0) and may be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, ligation and so forth, or stored until ready to use.

## V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACT****ME* **DNA BLOOD KIT** is tested using of standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

## VI. PRODUCT SPECIFICATIONS

| SAMPLE MATERIAL |
| --- |
| up to 1 ml of fresh or frozen blood, up to 200 µl plasma, serum, buffy coat, lymphocytes or body fluids |
| **EFFICIENCY** |
| 3–10 µg DNA from 200 µl of sample |
| **BINDING CAPACITY** |
| Approx. 27 µg DNA. |
| **TIME REQUIRED** |
| 25 minutes (including incubation time). |
| **DNA PURITY** |
| $A_{260}/A_{280}$ ratio = 1.7–1.9 |

## VII. SAFETY PRECAUTIONS

- → Biological samples are treated as a biohazardous material of its potential pathogen content or health and life-threatening substances. While working with biological material it is essential to comply with all safety requirements for working with biohazardous material.
- → It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- → Use of sterile pipette filter tips is recommended.
- → Avoid the cross contamination of DNA between columns.
- → Guanidine salts' residues may form highly reactive compounds when combined with oxidation components. In case of spillage, clean the surface with detergent water solution.

## VIII. RECOMMENDATIONS AND IMPORTANT NOTES

### DNA elution

An optimal volume of Elution Buffer used should be chosen in accordance with the quantity of sample material and final DNA concentration expected. Use of 50–100 µl of Elution Buffer when extracting from 100–500 µl of blood is recommended. Increasing Elution Buffer volume to 200 µl is recommended when extracting from 500–1000 µl of blood.

The quantity of purified DNA depends on the type of sample and the number of white blood cells it contains (patient's age, his health condition, sample transport conditions, as well as storage time and method). Usually, the isolation efficiency from 200 µl of blood of a healthy person is 3–10 µg of DNA. Higher quantities of DNA may be acquired from clinical samples containing increased number of white blood cells (3x $10^6$–1x $10^7$ cells/ml).

If a high DNA concentration is desired, the elution volume may be reduced to 20 µl. However, it may reduce the efficiency of the DNA retrieval. It is essential to apply Elution Buffer precisely to the centre of the membrane. In order to maximize DNA retrieval, heat Elution Buffer to 80°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, a second elution should be performed. For the second elution, repeat steps 18–21 of the Isolation Protocol (section XI), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

### Elution Buffer

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions. If desired, DNA can alternatively be eluted from the minicolumn with nuclease-free water (pH 7.0–9.0) or TE Buffer.

### RNA contamination

Co-extracted vestigial RNA contamination may interfere with some enzymatic reaction, but does not inhibit PCR. If an RNA-free DNA sample is desired, add 4 µl of RNase A solution (10 mg/ml; cat. no. RP14, RP45) to BL Buffer in step 5 of the Isolation Protocol. Mix well and incubate at 37°C for 5 minutes. After incubation, follow the Isolation Protocol from step 6 (section XI).

## IX. SAMPLE PREPARATION

When isolating from fresh blood, add the appropriate amount of an anticoagulant (EDTA, citrate) to a blood sample. If sample already contains an anticoagulant, skip this step.

A blood sample containing anticoagulant (EDTA, citrate) may be stored at +4°C (for no longer than 24 h) or frozen (a -80°C ultra-freezer is highly recommended). Avoid subjecting the blood sample to repeated freeze/thaw cycles before DNA isolation.

Mix the blood sample well by inverting the tube before collecting a blood volume for isolation. When using frozen blood, let it thaw completely over several minutes at room temperature.

In some cases, red blood cells may form a clot. Breaking the clot by thorough vortexing and/or pipetting the blood sample with RBC Lysis Buffer is recommended. The standard Isolation Protocol should then be followed.

Before transferring the lysate into a minicolumn (step 11), an additional step is required: 60 s of centrifugation at 11 000–21 000 x g, after which the supernatant should be transferred into the purification column. Avoid disturbing the pellet during the transfer.

When isolating from buffy coat or lymphocytes, do not use more than 5x $10^6$ cells. If less than 200 µl of sample is used, add Elution Buffer (10 mM Tris-HCl, pH 8.5; 0.5 mM EDTA) or PBS buffer (not included in the kit) to the 200 µl and follow the Isolation Protocol from step 1 (for blood and buffy coat samples) or step 5 (for plasma, serum, lymphocytes and body fluids).

## X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Prepare **Proteinase K** solution by reconstituting the lyophilizate in an appropriate amount of Proteinase Buffer.
3. Ensure that ethanol has been added to **BB**, **BW1** and **BW2 Buffers**. If not, add the appropriate quantity of **96–100% ethanol** (the volumes can be found on the bottle labels or in the table given in section II)
4. Examine the buffers. If a sediment has occurred in any of them, incubate it at **37°C** (**BB**, **BW1** and **BW2 Buffers**) or at **50–60°C** (other buffers), mixing occasionally until the sediment has dissolved. Cool to room temperature.
5. Set a dry block heater or water bath to **55°C**.
6. Unless otherwise stated, conduct all the isolation steps at room temperature.

## XI. ISOLATION PROTOCOL

When isolating from blood or buffy coat follow the Isolation Protocol from step 1, when isolating from plasma, serum, lymphocytes and body fluids start from step 5.

1. Transfer **200-1000 µl of sample** to a sterile 1.5–2 ml Eppendorf tube and add **the same volume** of **RBC Lysis Buffer** (for example, add 200 µl RBC Buffer to 200 µl sample).
   - ⚠ When isolating from less than 200 µl of sample, add Elution Buffer or PBS buffer to 200 µl and then add 200 µl of RBC Lysis Buffer.
2. Mix well by inverting the tube until a clear red solution is obtained.
3. Centrifuge for 4 min at 8600 x g.
   - ⚠ Higher speeds are not recommended as they may hinder the subsequent suspension of the white blood cell pellet in lysis buffer.
4. Carefully discard the supernatant from over the white blood cell pellet.
5. Add **375 µl BL Buffer**.
   - ⚠ When isolating from blood and buffy coat samples, resuspend the cell pellet completely.
   - ⚠ When isolating from plasma serum, lymphocytes and body fluids mix by inverting the tube .
6. Add **6 µl Proteinase K** and mix by vortexing.
7. Incubate at **55°C** for 10 min.
   - ⚠ If the pellet is not completely suspended in lysis buffer, extend the incubation time until the cells are completely lysed, vortexing every 60–120 s.
8. Add **400 µl BB Buffer** and mix thoroughly.
9. Vortex vigorously for 15–20 s.

10. Transfer the **lysate** onto a DNA Purification Column placed in a Collection Tube. Centrifuge for 60 s at 11 000–15 000 x g.
11. Transfer DNA Purification Column to a new Collection Tube (2 ml).
12. Add **600 µl BW1 Buffer** and centrifuge for 30 s at 11 000–15 000 x g.
13. Discard the filtrate and reuse Collection Tube.
14. Add **400 µl BW2 Buffer** and centrifuge for 30 s at 11 000–15 000 x g.
15. Discard the filtrate and reuse the Collection Tube.
16. Centrifuge for 60-120 s at 15 000–21 000 x g.
    ⚠ Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove alcohol completely from DNA Purification Column before elution.
17. Discard Collection Tube and the filtrate and carefully transfer DNA Purification Column to a sterile 1.5 ml Eppendorf microcentrifuge tube.
18. Add **50-100 µl Elution Buffer** directly onto DNA Purification Column membrane.
    ⚠ If a greater volume of Elution Buffer is to be used, follow the instructions in section VIII. Recommendations and important notes.
19. Incubate DNA Purification Column at room temperature for 120 s.
20. Centrifuge at 11 000–15 000 x g for 60 s.
21. Remove DNA Purification Column. Isolated DNA should be stored at **+4°C** or **-20°C** depending on further applications.

## XII. TROUBLESHOOTING

| Problem | Possible cause | Solution |
|---|---|---|
| **Some red blood cells are present in the white blood cell pellet.** | The red blood cells have not been completely lysed. | Discard the supernatant from over the white blood cell pellet and repeat RBC lysis step. However, if a small quantity of red blood cells remain, this will not interfere with the subsequent DNA isolation steps. |
| **A red blood cell clot was formed and cannot be lysed by RBC Lysis Buffer.** | Old material or improper material storage; also the source of the blood (for example, rat's blood). | Follow the procedure given in Section IX. Sample preparation (Red blood cell clot formation). |
| | Insufficient quantity of anticoagulant in the blood sample. | Collect the sample material again, doubling the amount of anticoagulant or follow the procedure given in Section IX. Sample preparation (red blood cell clot formation). |
| **The column becomes clogged during purification.** | Improper storage of the sample material. | Storing blood samples at -80°C is highly recommended. Avoid subjecting the samples to repeated freeze/thaw cycles. Store blood samples at +4°C no longer than 24 h. |
| | Incomplete white blood cell lysis. | Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 55°C, vortexing the lysate at several-minute intervals. |
| **Low yield of purified DNA.** | Material contains few white blood cells. | Increase sample volume and/or decrease Elution Buffer volume to 20 µl. |
| | Incomplete white blood cell lysis. | Resuspend the white blood cell pellet thoroughly and/or the extend incubation time at 55°C, vortexing the lysate at several-minute intervals. |
| | The purified DNA contains residual alcohol. | Repeat the isolation, paying a particular attention to whether any residual BW2 Buffer is left in DNA Purification Column after the final centrifugation step. |
| | Incomplete DNA elution from the membrane. | Before applying Elution Buffer to the membrane, heat it to 80°C. Apply Elution Buffer directly to the centre of the membrane. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl. |
| | The pH of the water used for elution is lower than 7.0. | Use Elution Buffer for DNA elution. |

| | | |
|---|---|---|
| **Purified DNA is degraded.** | Inappropriate sample storage conditions. | Storing blood samples at -80°C is recommended. Avoid subjecting the sample material to repeated freeze/thaw cycles. Store blood samples at +4°C no longer than 24h. |
| **Isolated DNA is of poor purity.** | Incomplete protein digestion due the reduced Proteinase K activity. | Prepare a fresh Proteinase K solution. Ensure Proteinase K solution is stored at -20°C. |
| | One of the washing steps was omitted. | Repeat the isolation, performing both washing steps. |
| | Purified DNA contains residual alcohol. | Repeat the isolation, paying a particular attention to whether any residual BW2 Buffer is left in DNA Purification Column after centrifugation in step 16. |
| **RNA contamination present.** | Both DNA and RNA were bound to membrane. | If RNA could interfere in downstream applications, the additional RNase A step should be included (see Section VIII. Recommendations and important notes). |

## XIII. SAFETY INFORMATION

### RBC Lysis Buffer

**Warning**
H319, H412
P264, P273, P305+P351+P338

### Proteinase K (lyophilized)

**Danger**
H315, H319, H334, H335
P261, P271, P304+P340, P342+P311, EUH208

### BL Buffer

**Warning**
H319
P264, P305+P351+P338

### BB Buffer

**Danger**
H318, H302, H332, H315, H412
P280, P261, P305+P351+P338, P301+P312 P330, P304+P340 P312

### BW1 Buffer

**Danger**
H318, H315, H412
P280, P305+P351+P338 P310

**EUH208:** Contains Proteinase. May produce an allergic reaction. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H332** Harmful if inhaled. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **H412** Harmful to aquatic life with long-lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P271** Use only outdoors or in a well-ventilated area. **P273** Avoid release to the environment. **P280** Wear protective gloves/protective clothing/ eye protection/face protection. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P342+P311** If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. **P304+P340** IF INHALED: Remove person to fresh air and keep comfortable for breathing. **P312** Call a POISON CENTER/ doctor if you feel unwell. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. **P310** Immediately call a POISON CENTER/doctor.