

Instructions for use

EXTRA-GENE I

Kit for the isolation of genomic DNA
50 Extractions

REF 7059



1. Product description

EXTRA-GENE is a kit for fast extraction of genomic DNA without the usage of organic solvents. The kit contains all reagents required for the isolation of 50 single samples. The isolation is based on a selective erythrocyte lysis which is followed by a detergent break down step with subsequent salting out of the proteins [1] and purification of DNA by precipitation. In less than 60 minutes, DNA is extracted without the need to prepare any reagents or solutions. The preparation has a purity index R (extinction ratio OD_{260}/OD_{280}) sufficient for the use in enzymatic reactions [2]. Amplification inhibitors, e.g. ferrum from haemoglobin [3], are removed effectively. When the DNA is applied in the PCR★ EDTA or citrate blood should be used, since Heparin strongly inhibits amplification [4]. The yield is approximately 5-30 μg of high molecular weight DNA from 500 μl of whole blood with a normal leucocyte concentration (see 3.3). Extraction of DNA from cell culture material and purified lymphocytes is also possible with the EXTRA-GENE I kit. Without further purification, the DNA can be used in restriction analyses (RLFP), in Southern Blot techniques or in the PCR★.

2. Material

2.1. Contents of the kit

- ◆ 2x 50 ml **Solution 1** (erythrocyte-lysis-buffer)
- ◆ 1x 10 ml **Solution 2** (extraction-buffer)
- ◆ 2x 10 ml **Solution 3** (protein-precipitation-reagent)
- ◆ Instructions for use

2.2. Additional material and equipment required

- ◆ Eppendorf reaction tubes; 1.5 ml / 2.0 ml; sterile
- ◆ Pipettes (20 µl- 1000 µl)
- ◆ Centrifuge (at least 13.000 rpm)
- ◆ Heatingblock (56°C) or water bath
- ◆ Vortex mixer
- ◆ Ethanol 96%
- ◆ Ethanol 70%
- ◆ Aqua dest., sterile

2.3 Storage and stability

All reagents should be stored at 2...8°C. The expiration date is indicated on the package.

3. Method

3.1 Preparation

Solution 2 will go cloudy when stored below room temperature. This means no loss of quality. Before use, resolve precipitated components by incubation at **37°C**.

DNA extraction is optimized to 500 - 600 µl of blood per isolation. If possible, do not exceed or fall below this quantity. If larger quantities of DNA are required, do several parallel isolations.

For use in the PCR take EDTA or citrate blood only!

If purified lymphocytes or leucocytes, respectively cell culture material is used, begin extraction with ↵ (starting with sedimented cells). Use a maximum of 10^7 cells per isolation.

3.2 DNA isolation

- ◆ Add **900 µl of Solution 1** to 0.5 - 0.6 ml of blood in a 1.5 ml reaction tube and shake briefly. Centrifuge for 1 minute at 8000 rpm.
- ◆ Discard the supernatant and wash the sediment with **1 ml of Solution 1**. Centrifuge for 1 minute at 8000 rpm.
- ◆ Discard the supernatant and
↪ resuspend the leucocyte sediment in **240 µl aqua dest.**
Add **120 µl Solution 2** and shake or vortex until the mixture is clear.
- ◆ Add **120 µl Solution 3**.
Vortex thoroughly and incubate for 5 minutes at room temperature.
Centrifuge for 5 minutes at 13 000 rpm.
- ◆ Transfer the supernatant into a new 1.5 ml reaction tube.
Add **120 µl Solution 3**.
Vortex thoroughly and incubate for 5 minutes at room temperature.
Centrifuge for 5 minutes at 13 000 rpm.
- ◆ Transfer the supernatant to a new reaction tube.
Add **1 ml of ethanol 96%** and mix by gently turning the reaction tube.
Centrifuge for 2 minutes at 13 000 rpm.
- ◆ Carefully remove the supernatant and discard it. Add **1 ml of ethanol 70%** and shake briefly.
Centrifuge for 2 minutes at 13 000 rpm.
- ◆ Carefully decant the supernatant and discard it. Place the reaction tube with opening down on a filter paper and let it air dry for about 5 minutes.
- ◆ Resolve the DNA pellet in 100 µl of aqua dest. (resuspend using a pipette). If complete DNA dissolution is difficult warm up to 56°C for about 10 minutes.
- ◆ Determine DNA concentration and purity if desired.
- ◆ Use DNA for further tests or store at $\leq -20^{\circ}\text{C}$.

3.3 Determination of DNA concentration and purity

Determination of concentration or purity is not necessary if there is a normal number of leucocytes. Out of 500 - 600 µl of blood an average DNA quantity of 5-30 µg is obtained in this case. With deviating numbers of leucocytes, the DNA yield can be estimated according to the following table:

average number of leucocytes x10 ³ per µl (approx.)	2	3	5	10	20	30
Yield of DNA in µg (approx.)	1-5	2-8	5-10	10-20	20-40	30-60

For leucocyte numbers >30x10³ / µl use the half amount of blood.

The DNA concentration can be determined by measuring absorption at 260 nm. For genomic (double-stranded) DNA, concentration can be estimated according:





$$1 \text{ OD}_{260} = 50 \text{ µg/ml}$$

Using absorption measurement at 260 and 280 nm, the degree of purity of the DNA can also be determined. For pure DNA an OD₂₆₀ / OD₂₈₀ ratio is applicable between 1.6 and 2.0. Higher values indicate the existence of RNA, lower values mean contamination with protein.

4. References

- [1] Miller, S. A., et al., 1988. Nucleic Acid Res. **16**:1215
- [2] Allen, F. S., et al., 1972. Biopolymers **11**:853
- [3] Singer-Sam, J., et al., 1989. Amplification **3**:11
- [4] Beutler, E., et al., 1990. BioTechniques **9**:166

5. Explanation of symbols used on Labelling

	Storage temperature		Batch code
	Use by		Consult instructions for use
REF	Catalogue number		

★ The Polymerase Chain Reaction (PCR) is covered by patent rights. Commercial use of this method in in-vitro diagnostics requires a licence from Hoffmann-LaRoche, Basel. BAG emphasizes, that the user is responsible for legitimate use of PCR.



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