EN

Instructions for use

EXTRA-GENE I

Kit for the isolation of genomic DNA
50 Extractions

REF 7059

1. Product description

EXTRA-GENE I 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 detergence break down step with subsequent salting out of the proteins [1] and purification of DNA by precipitation. In less than 60 minutes, DNA can be extracted without the need to prepare any reagents or solutions. The resulting DNA 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 - 600 µl of whole blood with a normal leucocyte concentration. 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 EXTRA-GENE I 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

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2.2. Required additional material and equipment

- 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

3. Storage and stability

The EXTRA-GENE I kit is delivered at ambient temperature. Store all reagents at 2…8°C upon receipt. The expiry date is indicated on the label of the package and each reagent and is also valid for opened reagents.

4. DNA extraction

4.1 Preparation

Solution 2 can be turbid when stored below room temperature, but this does not influence the quality. Resolve precipitated components by incubation at 37°C before usage.
DNA extraction is optimized for 500 - 600 µl of blood per isolation. If possible, do not exceed or fall below this quantity (exception see 4.3). If larger quantities of DNA are required, do several isolations in parallel. For use in the PCR use EDTA or citrate blood only!
If purified lymphocytes or leucocytes, respectively cell culture material is used, start extraction with ↳ (starting with sedimented cells). Use a maximum of 10^7 cells per isolation.

4.2 DNA extraction

- Add 900 µl of **Solution 1** to 0.5 - 0.6 ml 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** (shake manually). 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 solution 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 into a new reaction tube.
  Add **1 ml ethanol (96%)** and mix by gently turning the reaction tube.
  Centrifuge for 2 minutes at 13 000 rpm.
- Carefully decant the supernatant and discard it. Add **1 ml 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 dry for about 5 minutes.
- Resolve the DNA pellet in 100 µl aqua dest. (use a pipette for resuspending).
  If the DNA is difficult to solve warm up to 56°C for approx. 10 minutes.
- Determine DNA concentration and purity if desired.
- Determination of DNA concentration and purity see 4.3.
- Use DNA for tests or store at ≤ -20°C.

4.3 Determination of DNA concentration and purity

Determination of concentration or purity is not necessary if there is a normal number of leucocytes. With 500 - 600 µl blood an average DNA quantity of 5-30 µg is obtained. 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 >30 x 10^3/ 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 OD_{260} = 50 µg/ml

By absorption measurement the degree of purity of the DNA can also be determined.
DNA should have the following purity indexes:

- OD_{260}/OD_{280} = >1.5 and <2.0 (indicator for contamination with RNA/proteins)
- OD_{260}/OD_{230} = >1.8 (indicator for contamination with salt, carbohydrate or organic solvents)

5. Warnings and Precautions

Biological material used for extraction of DNA, e.g. blood or human tissue, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

EXTRA-GENE I contains no hazardous substances in concentrations requiring labeling.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

6. 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

7. Explanation of symbols used on Labelling

	Storage temperature	LOT	Batch code
	Use by		Consult instructions for use
REF	Catalogue number	IFU	Instructions for use
CONT	Content, contains	DNA EXTRACTION	Intended use: DNA extraction
SOLN | 1	Solution 1	CONT | BUF | LYSIS	Contain erythrocyte lysis buffer
SOLN | 2	Solution 2	CONT | BUF | EXTRACTION	Contain extraction buffer
SOLN | 3	Solution 3	CONT | REAG | PRECIPITATION	Contain protein precipitation reagent

Instructions for use in other languages see:
http://www.bag-healthcare.com
http://service.bag-healthcare.com
or phone: +49 (0)6404-925-125

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