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DNA isolation kit for Isolation of DNA from Reaction Mixtures

Cat. No. DR-50, DR-250

Important!

We are constantly improving the protocol for the kit. Please use the protocol provided with the product.

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Description

The kit is designed for isolation and purification of DNA (from 50 to 10000 base pairs) from reaction mixtures. For instance, it is possible to purify DNA from dNTP, enzymes, unbound low molecular weight radioactive and fluorescent labels, etc. The method of DNA isolation is based on the selective binding of nucleic acids from a lysed sample on a silica-gel membrane, followed by washing and the elution of the purified sample. It is possible to isolate up to 10-20 µg of DNA.

The isolated DNA can be used for PCR, transcription, nick-translation, sequencing, and other genetic engineering applications.

The kit does not contain phenol and chaotropic salts like guanidine thiocyanate.

Contents

	DR-50 50 preps	DR-250 250 preps
Preparation buffer PB (concentrate)	12 ml	2x30 ml
Wash buffer WB (concentrate)	6 ml	2x14ml
Elution buffer EB	2x5 ml	50 ml
Collection tubes and spin columns	50 pcs	250 pcs

Safety information

Caution! When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. You must follow the rules of general and personal safety when working with the kit.

Equipment and reagents to be supplied by user

- Microcentrifuge with rotor for 1.5 ml tubes, speed 10000 rcf
- 1.5 ml microcentrifuge tubes
- Ethanol, 96-100%

Before starting the procedures

Preparation buffer PB. Add ethanol (95-99 %) to PB buffer and mix.

- 50 preps. To obtain 40 ml of PB buffer, add 28 ml of ethanol to 12 ml of PB buffer (concentrate).
- 250 preps. To obtain 100 ml of PB buffer, add 70 ml of ethanol to 30 ml of PB buffer (concentrate).

Wash buffer WB2. Add ethanol (95-99 %) to WB buffer and mix.

- 50 preps. To obtain 30 ml of WB buffer, add 24 ml of ethanol to 6 ml of WB buffer (concentrate).
- 250 preps. To obtain 70 ml of WB buffer, add 56 ml of ethanol to 14 ml of WB buffer (concentrate).

Note! It is recommended to add ethanol to the aliquots of the WB buffer, since ethanol may partially evaporate when storing the buffer for several months.

DNA isolation protocol

1. Add 5 volumes of PB buffer to an aliquot of the sample. For example, add 100 µl of PB buffer to 20 µL of the sample.

2. Mix the sample and apply to the column. Centrifuge the column at 10000 rcf for 30 sec. Discard the flow-through.

Note: The column volume is 800 µL. If the volume of the mixture in point 1 is more than 800 µl, apply the excess to the same column and repeat point 2.

3. Apply 500 µl of WB buffer to the column. Centrifuge the column at 10000 rcf for 30 sec. Discard the flow-through.

4. Centrifuge at 10.000 rcf for 3 min to completely remove the WB buffer.

5. Transfer the column into a new 1.5 ml microcentrifuge tube (not included in the kit).

6. Apply 60-100 µl of EB buffer to the center of the column membrane. Incubate for 3 min at room temperature (20-25 °C). Centrifuge the column at 10000 rcf for 30 sec.

Note: The EB buffer contains 10 mM Tris • HCl (pH 8.0). The sample can also be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0-8.5) or water (pH 8.0-8.5). EDTA may inhibit following enzymatic reactions.

7. Store the eluate containing DNA at -20 °C.

Analysis of isolated DNA.

DNA can be analyzed can be analyzed by gel electrophoresis in agarose or polyacrylamide gel.

The amount of isolated DNA can be estimated by using UV spectrometry.
The maximum of absorption for DNA corresponds to λ = 260 nm.
DNA concentration (µg/ml) can be calculated using the following formula:
A_{260} * dilution * 50 µg/ml.
Typical optical density ratios are A_{260}/A_{280} ~1.7-2.0.

Storage

All components of the kit can be stored at room temperature (15-25 °C) for up to 12 months.

Shipping

All components of the kit are shipped at room temperature.