Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

Kit for isolation of DNA/RNA from animal and bacterial cells, swabs, viruses

Cat. No. PN-100

Important!

We are constantly improving the protocol for the kit. Please use the protocol provided with the product.
Updated March 2022

Description

The kit is designed for DNA/RNA isolation and purification from animal and bacterial cells cultures, swabs, viruses. Lysis buffer lyses cell membrane releasing nucleic acids. Subsequent steps are precipitation and washing of nucleic acids (DNA/RNA) followed by dissolving of samples.

Note: Lysis buffer contains nucleic acids co-precipitant, therefore using additional co-precipitant reagent using isn't necessary.

Important! RNase or DNase treatment is required if clean DNA or RNA is needed.
The isolated DNA can be used for PCR, nick-translation, sequencing and other genetic engineering applications.

The isolated RNA can be used for RT-PCR, sequencing and other genetic engineering applications.

Kit Components

	PN-100 100 preps
Lysis buffer LB	70 ml
Precipitation buffer PB	100 ml
Wash buffer WB2 (concentrate)	22 ml
Suspension buffer SB	10 ml

Safety Precautions

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. Lysis buffer LB contains guanidine thiocyanate solution, which is irritating and toxic. Buffer are toxic in contact with skin and insides, causing burns. In case of a contact with skin, wash immediately with plenty of water and detergents. Visit a doctor if necessary.

CAUTION! Precipitation buffer PB isopropanol, which is irritating and toxic. Don't work with this buffer near the open fire.

Equipment and Reagents to be Supplied by User

- Microcentrifuge with rotor for 1.5 ml tubes, speed 12000 rcf
- Water bath or heating block at 65 °C
- 1.5 ml microcentrifuge tubes
- Ethanol, 96-100%

Before starting the procedures:

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

- 1 prep. To obtain 500 µl of WB buffer, add 400 µl of ethanol to 100 µl of WB buffer (concentrate).
- 100 preps. . To obtain 110 ml of WB buffer, add 88 ml of ethanol to 22 ml of WB buffer (concentrate).

To isolate RNA from gram-positive bacteria, prepare a solution of lysozyme with a concentration of 50 mg/ml in TE buffer, pH 8.0 (0.01 M Tris-HCl (pH 8.0), 0.001 M EDTA (pH 8.0)).

DNA/RNA isolation protocol.

Sample lysis

1) Animal or bacterial cells

Add 600 µl of LB buffer to the cell pellet. Mix thoroughly by pipetting, avoiding foaming. Incubate at 65 °C for 10 minutes.

Note: when isolating DNA or RNA from gram-positive bacteria, add 30 µl of lysozyme solution (50 mg / ml) in TE buffer, pH 8.0 (0.01 M Tris-HCl (pH 8.0), 0.001 M EDTA (pH 8.0)).

Note: Do not use more than $5*10^6$ animal cells and more than $1*10^8$ bacterial cells.

2) Epithelial cells swabs. Nasal or throat swabs

Take an aliquot with a volume of 100 µl of saline or transport medium after incubation of a cotton swabs from nose or mouth. Add 500 µl of LB buffer. Mix thoroughly by pipetting, avoiding foaming. Incubate at 65 °C for 10 minutes.

DNA/RNA precipitation

1) Add 900 µl of PB buffer to 600 µl of lysate. Mix thoroughly by pipetting or vortexing.
2) Centrifuge at 12000 rcf for 30 sec. Discard the supernatant carefully avoiding disturb the precipitate.

DNA/RNA precipitate washing

1) Add 500 µl of WB buffer to the precipitate. Mix gently by hand turning the tube upside-down 3-5 times.

Note: Ensure that ethanol was added to the WB buffer.

2) Centrifuge at 12000 rcf for 30 sec. Discard the supernatant carefully avoiding disturb the precipitate.
3) Repeat point 1 and 2 at "DNA/RNA precipitate washing" section.

DNA/RNA dissolving

1) Dry the precipitate at 65 °C for 10 minutes on air.
2) Add 50 µl of SB buffer to precipitate.
3) Mix thoroughly by pipetting or vortexing. Incubate at 65 °C for 5 minutes

Note: SB buffer is RNase-free DEPC-treated water.

4) Store the eluate containing RNA at -20 or -80 °C.

Note: For the best result it's recommended to use RNA for RT-PCR at the isolation day. During the day RNA solution can be stored at +4 °C.

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.