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DNA/RNA Prep

nucleic acid extraction kit for the extraction and purification of total RNA/DNA from clinical materials

USER MANUAL

REF K-2-9

Σ 50

NAME
DNA/RNA Prep

INTENDED USE
The **DNA/RNA Prep** nucleic acid extraction kit is intended for the extraction and purification of total RNA/DNA from clinical materials (peripheral blood plasma, cerebrospinal fluid, amniotic fluid, nasal and throat swabs, saliva).

PRINCIPLE OF ASSAY
DNA/RNA Prep nucleic acid extraction kit is reagents kit for rapid and efficient manual extraction and purification of DNA/RNA from various biological materials. Lysis Sol contains chaotropic agent (guanidine thiocyanate) that lyses cells and denaturates cell proteins. The nucleic acids are then precipitated in isopropanol. The nucleic acids are eluted in low salt buffer and are ready-for use in subsequent reactions. The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic manipulation. We highly recommend the use of internal standards as well as positive and negative controls in order to monitor the purification, amplification and detection processes.

MATERIALS PROVIDED
- **Lysis Sol**, 15 ml;
- **Prec Sol**, 20 ml;
- **Washing Sol 3**, 25 ml;
- **Washing Sol 4**, 10 ml
- **RE-buffer**, 4 x 1,2 ml;

Contains reagents for 50 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
- Biological cabinet
- Vortex
- Tube racks
- Desktop microcentrifuge for “eppendorf” type tubes
- 65°C ± 2°C dry heat block
- Microcentrifuge tubes, 1,5 - 2,0 ml
- Pipettes
- Sterile, RNase-free pipette tips with filters
- Biohazard waste container
- Disposable gloves, powderless
- Refrigerator
- Freezer

WARNINGS AND PRECAUTIONS

-  Xn

  Component Lysis Sol contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/38; S: 36/37/39).

  Risk Phrases

  R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed

  R 22 Harmful if swallowed

  R 36/38 Irritating to eyes and skin

  Safety Phrases

  S 13 Keep away from food, drink and animal feedstuffs

   X

  Component Prec Sol contains 2-propanol: flammable. Irritant. (R10-36-67, S7-16-24/25-26)

  Risk Phrases

  R10: Flammable

  R36/37/38: Irritating to eyes, respiratory system and skin

  R67: Vapors may cause drowsiness and dizziness

  Safety Phrases

  S7: Keep container tightly closed

  S16: Keep away from sources of ignition - No smoking

  S24/25: Avoid contact with skin and eyes

  S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;

- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

SPECIMEN COLLECTION AND CONSERVATION

DNA/RNA-prep nucleic acid extraction kit is recommended for **RNA and DNA** extraction and purification from:

- *whole blood*;
- *serum*;
- *plasma*;
- *bone marrow aspirate*;
- *cerebrospinal fluid (liquor)*;
- *amniotic liquid*;
- *sinovial liquid*;
- *peritoneal and pleuric versament*;
- *tissue* homogenized with mechanical homogenizer and dissolved in PBS sterile;
- *urine (sediment)*;
- *prostatic liquid*;
- *seminal liquid*;
- *swabs;*
- *sputum:*
    - Collect sputum into 50 mL single-use PP tubes with a screw cap.
    - In a biological safety cabinet, homogenize samples after mixing with equal volume of 4% NaOH solution. (*N-acetyl-L-cysteine may be added if required in the amount of 50-70 mg per sample).* Mix intensely with a tube rotator for 5-20 minutes (depending on the density of a sample).
    - Centrifuge samples at 3000 rpm (2800-3000 g) for 15 min and carefully discard the supernatant leaving 500-1000 µl in the tube. Resuspend sediment and transfer it into a 1.5 ml tube.
    - Centrifuge samples at 12000 rpm for 5-10 min, discard the supernatant and use the same 1,5 ml sample tube for DNA isolation from sample sediment.
- *bronco aspirate:* transfer 1,0 ml to a polypropylene tube (1,5 ml) and centrifuge at 10000g/min for 10 min. Discard the supernatant and leave about 100 µl of solution for DNA extraction;
- *feces:*
    - Prepare 10-20% feces suspension, for instance adding 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces in 5 ml tube (the same can be done in 2,0 ml tube). The DNA/RNA purification must be done immediately, if it is not possible add 20% Glycerol sterile solution (cryoprotective agent that provides intracellular and extracellular protection against freezing) and store at -20°C.
    - Vortex to get an homogeneous suspension and centrifuge for 5 min to 7000-12000g. Use the supernatant for the extraction of the viral DNA/RNA and the bacterial fraction (white-yellowish line between the sediment and the supernatant) for the extraction of bacterial DNA.

All kind of biological fluids or semi-fluid samples can be processed. For successful nucleic acid purification, it is important to obtain a homogeneous, clear and non-viscous sample before loading into the corresponding isolation tube. Therefore, check all samples (especially old or frozen ones) for the presence of precipitates.
Note: Handle all specimens as if they are potentially infectious agents.
It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

STORAGE CONDITIONS AND PREPARATION OF WORKING SOLUTIONS

- **DNA/RNA-prep** kit should be stored dry at +2-8°C; storage at higher temperatures should be avoided.
- If crystals are observed Sol Lys reagent bottle upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear or prewarm at 60°C for a maximum of 5 min in order to redissolve salts.

STABILITY

DNA/RNA-prep is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. All components of the RIBO-prep nucleic acid extraction kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

PROTOCOL

1. Prepare required number of 1.5 ml disposable polypropylene micro centrifuge tubes including one tube for Negative Control of Extraction (**Negative Control**, **C-**) and one tube for Positive Control of Extraction (**Positive Control** (RNA or DNA), if provided with the amplification kit).
2. Add to each tube **10 µl** of **Internal Control** (if provided with the amplification kit) and **300 µl** of **Lysis Sol**
3. Add **100 µl** of samples to the appropriate tubes using pipette tips with aerosol barriers.
4. Prepare Controls as follows:
    - add **100 µl** of **C–** (**Neg Control** provided with the amplification kit) to the tube labeled C*neg*
    - add **90 µl** of **Negative Control** (provided with the amplification kit) and **10 µl** of **Positive Control** to the tube labeled C*pos*.
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 7-10 sec.
6. Add **400 µl** of **Prec Sol** and mix by vortex.Centrifuge all tubes at 13,000 r/min for 5 min and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
7. Add **500 µl of Wash Sol 3** into each tube. Vortex vigorously to ensure pellet washing. Centrifuge all tubes at 13,000 r/min for 60 sec and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
8. Add **200 µl of Wash Sol 4** into each tube. Vortex vigorously to ensure pellet washing. Centrifuge all tubes at 13,000 r/min for 60 sec and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
9. Incubate all tubes with open caps at **65 °C for 5 min**.
10. Resuspend the pellet in **50 µl of RE-buffer** (elution volume can be increased up to 90 µl). Incubate for 5 min at 65°C and vortex periodically.
11. Centrifuge the tubes at 13000g for 60 sec.

The supernatant contains RNA/DNA ready for amplification. If amplification is not performed the same day of extraction, the processed samples can be stored at 2-8°C for at maximum period of 5 days or frozen at - 20°/-80°C.

TROUBLESHOOTING

These troubleshooting rules may be helpful in explaining any questions that may arise.

False negatives with extraction product:

- Degradation of the nucleic acid contained in the sample. It's necessary to use a new sample. Store samples under appropriate conditions. Use plastic free from DNAses and RNAses
- Loss of pellet. Carefully draw off the washing solutions and try not to remove the nucleic acid residue.

False positives with extraction product:

- Contamination during sample extraction. Open one test tube at time. Avoid spilling the contents of the test tube, always change tips. Use only filter tips during the extraction procedure. Change tips between tubes.
- Contamination of the reagents prepared for the step. Repeat the test with the new set of reagents.
- Contamination of the extraction zone by amplicons. Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol, wash lab coats, replace test tubes and tips in use. Use different laboratory coats in different Amplification areas.

Key to symbols used

	List Number		Caution!
	Lot Number		Contains sufficient for <n> tests
	For *in Vitro* Diagnostic Use		Version
	Store at		Expiration Date
	Manufacturer		Flammable
	Consult instructions for use		Harmful

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