Magnetic DNA/RNA Extraction kit

Kit for DNA/RNA extraction based on
guanidine thiocyanate lysis and sorption to magnetic beads

Instructions for use: ***page 16***

IVD

80-06 REF

Σ *100 Extraktionen*
100 extractions

2. INTENDED USE

Magnetic DNA/RNA Extraction kit is designed for simultaneous isolation of viral, bacterial, and human DNA and RNA from clinical samples: blood plasma, saliva, swabs (nasopharyngeal, buccal, and urogenital), and urine for further nucleic acid amplification testing including Real-Time PCR and RT-PCR. The Kit is designed for 100 extractions.

3. PRINCIPLE OF THE TEST

Magnetic DNA/RNA Extraction kit uses the principle of nucleic acids binding to surface of magnetic beads, with subsequent washing steps and elution of purified nucleic acids.

4. MATERIALS PROVIDED

LB	**Lysis buffer**	1 bottle - 30 mL
SS	**Sorption solution**	1 bottle - 30 mL
W1	**Washing solution 1**	3 bottles - 3x50 mL
W2	**Washing solution 2**	1 bottle - 50 mL
W3	**Washing solution 3**	1 bottle - 20 mL
EB	**Elution buffer**	1 bottle – 10 mL

NC	**Negative control**	1 microcentrifuge tube - 1.5 mL
MBS	**Magnetic Beads Suspension**	1 microcentrifuge tube - 1.1 mL

Note: *If extracted DNA/RNA is intended for the subsequent analysis with Astra Biotech Infectious PCR kits, internal control* **IC** *contained in these PCR kits should be used for the extraction procedure with the* ***Magnetic DNA/RNA Extraction kit****.*

5. EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- Biosafety cabinet;
- Internal Control **IC** from the respective PCR diagnostic kit (optional);
- Heating block or thermo-shaker (temperature up to +65 °C) suitable for 1.5 mL tubes;
- Vortex mixer (maximum speed 2,400 rpm);
- Magnetic separation stands;
- Microcentrifuge tubes rack;
- Microcentrifuge (max. speed 13,000 rpm) suitable for 1.5 mL tubes (for sample preparation only);
- Medical aspirator;

- Non-filter pipette tips (up to 200 µL) for use with medical aspirator;
- 2 separate sets of pipettes for DNA-free and DNA-containing components (20-200 µL; 100-1,000 µL);
- Filter tips (100; 200 and 1,000 µL);
- Disposable 1.5 mL microcentrifuge tubes, RNase/DNase free;
- 15 mL conical centrifuge tubes;
- DNA-zone-only working area with separate protective laboratory clothing;
- Waste bin with disinfectant;
- 0.9 % sodium chloride solution and transport medium.

6. STORAGE CONDITIONS AND STABILITY OF THE KIT

The expiry date of the kit is stated on the box label, expiry date for each component is indicated on the respective label. **Magnetic DNA/RNA Extraction kit** can be stored in the original kit box at +2…+25 °C during the entire shelf life.

7. SAMPLE COLLECTION AND STORAGE

Type	Conditions	Stability time
Blood plasma	+2…+8 °C	5 days
	-18…-22 °C	1 year
	-68…-72 °C	Longtime stability
Swabs and urine samples	+18…+25 °C	2 days
	+2…+8 °C	4 days (2 weeks for urine)
	-18…-22 °C	Longtime stability
Saliva	+18…+25 °C	6 hours
	+2…+8 °C	1 week
	-18…-22 °C	1 month
	-68…-72 °C	Longtime stability

8. SAMPLE PREPARATION

- **Urine samples**

Shake the urine specimen cup. Transfer 1 mL of urine into new 1.5 mL microcentrifuge tube. Centrifuge at 13,000 rpm for 5 min. Remove the supernatant using medical aspirator. Add

0.9 % NaCl solution to obtain overall volume of 200 µL. Vortex thoroughly until pellet dissolves.

- **Saliva-Mouthwash samples**

Rinse mouth thoroughly with 10 mL of 0.9 % sodium chloride solution during 10-15 s. Collect fluid in sterile 15 mL microcentrifuge tube. Centrifuge at 3,000 rpm for 3 min. Remove approximately 9 mL of the supernatant using medical aspirator. Residual volume should be approximately 0.5-1 mL (pellet and liquid phase). Add 200 µL of transport medium. Vortex thoroughly until pellet dissolves.

- **Swabs and scrapes samples**

Collect the swabs with a suitable tool. After specimen collection place swab into a capped 1.5 mL microcentrifuge tube with transport medium and rotate it for 15 s. Avoid splashing the solution. Squeeze out residual liquid from the swab and discard the swab.

- **Blood plasma**

To obtain blood plasma centrifuge whole blood with anticoagulant at 3,000 rpm for 20 min at room temperature (+18…+25 °C). Transfer the supernatant into 1.5 mL microcentrifuge tube.

9. ASSAY PROCEDURE FOR DNA/RNA EXTRACTION

Note: *In case of **no** subsequent analysis with Astra Biotech Infectious PCR kits is followed; skip steps D, E and G.*

A. Preheat a heating block to +65 °C.

B. Check the Lysis buffer **LB** for precipitation. If **LB** forms a precipitate, it should be heated until the precipitate dissolves. Shake the bottle briefly and allow cooling to room temperature before use.

C. Prepare and label required quantity of 1.5 mL microcentrifuge tubes equal to the number of samples.

Note: *In case of subsequent analyses with Astra Biotech Infectious PCR kits: take one 1.5 mL microcentrifuge tube for negative extraction control and label as "NEC". **NC** must be extracted simultaneously with clinical samples in "NEC" tube.*

D. Vortex thoroughly Internal control **IC** tube then centrifuge briefly for 2-3 s at 1,500-2,400 rpm.

E. Add 20 µL **IC** into each prepared microcentrifuge tube (including "NEC").

F. Add 300 µL **LB** into each prepared microcentrifuge tube.

G. Add 200 µL **NC** to the microcentrifuge tube marked as "NEC".

H. Add 200 µL of clinical sample to microcentrifuge tubes.

I. Briefly vortex for 3-5 s and spin down by short centrifugation.

J. Incubate at +65 °C for 10 min. Vortex tube every 2 min for 1-2 s. If thermo-shaker is used, set up 1,300 rpm.

K. Transfer tubes back to the rack, cool for 2-3 min, and then spin down by short centrifugation.

L. Mix **MBS** by vortexing until magnetic beads completely resuspended.

M. To each tube add 300 µL **SS** and 20 µL **MBS**, briefly vortex and then let stand for 5 min.

N. Briefly vortex again and place in magnetic stand for 3 min. Ensure that magnetic beads completely pelleted to the tube wall against magnet.

O. Carefully aspirate supernatant, avoiding touching magnetic beads pellet.

P. Immediately transfer tubes in the rack and add 700 µL **W1** by pouring it over magnetic beads pellet to wash it from the wall, and then vortex to completely resuspend magnetic beads.

Q. Place tubes in magnetic stand for 2 min. Ensure that magnetic beads pelleted to the tube wall against magnet.

R. Carefully aspirate supernatant, avoiding touching magnetic beads pellet.

S. Repeat steps **P-R**.

T. Repeat steps **P-R** using 500 µL **W2**.

U. Repeat steps **P-R** using 200 µL **W3**.

V. After last wash, leave tubes on magnetic stand and air-dry magnetic beads pellet for 4 min with the tubes lids open. **Do not allow the pellet to overdry!**

W. Transfer tubes in rack, add 100 µL **EB**, briefly vortex, spin down by short centrifugation and then incubate in heat block at +65 °C for 5 min.

X. Transfer tubes to rack, cool for 2-3 min, briefly vortex and spin down by short centrifugation.

Y. Place tubes in magnetic stand for 2 min. Ensure that magnetic beads pelleted to the tube wall against magnet.

Z. Avoiding touching magnetic beads pellet, carefully collect supernatant, containing purified nucleic acids, and transfer it to fresh microcentrifuge tubes. It is recommended to use 5 µL for PCR reaction with RNA reverse transcription step, and 10 µL for PCR with DNA as template.

Note: *For prolonged use, store as recommended:*

For RNA: at +2 … +8 ^{o}C for up to 4 hrs; at -20 ^{o}C for up to 1 months; at -70 ^{o}C for up to 1 year;

For DNA: at +2 … +8 ^{o}C for up to 7 days; at -20 ^{o}C for up to 1 year.

10. TROUBLESHOOTING

1. <u>Low yield of DNA/RNA</u>

- Volume of added specimen is too large. Do not exceed volumes designated in this manual.
- Specimen may be old or degraded. Store specimens appropriately or use fresh specimens.
- Specimen inefficiently disrupted. Warm Lysis buffer until the precipitate dissolves completely.
- Pellet is dissolved incompletely. Do not overdry pellets.

2. Downstream applications are inhibited

- Residual Washing solutions in purified DNA/RNA: Carefully remove all Washing solutions as much as possible then dry pellets as consisted with procedure.

3. DNA/RNA is degraded

- Sample was undergone repeated frozen-thaw cycles. Avoid repeated frozen-thaw cycles, use fresh preparations immediately.
- Inappropriate storage conditions. Store samples and components as consisted with protocols.

11. PERFORMANCE CHARACTERISTICS OF THE ASSAY

Expected yields of genomic DNA/RNA will vary depending on the amount and type of starting material used. The efficiency of extraction is not less than 85 %.

12. QUALITY CONTROL

The quality of obtained DNA/RNA with the kit can be assessed:

- by analysis of internal control in case of subsequent analyses with Astra Biotech Infectious PCR kits;
- by DNA/RNA gel electrophoresis on a 1 % agarose.

13. SAFETY PRECAUTIONS

The operator should thoroughly follow the manual to obtain the reliable data.

Lysis buffer **LB** , Sorption solution **SS** , Washing solution 1 **WB1** , Washing solution 2 **WB2** and Washing solution 3 **WB3** are harmful, irritant and can cause serious eye damage; the following precautions should be observed:

- Wash the hands thoroughly after handling.
- Do not eat, drink or smoke when using this product.
- Wear protective gloves/ protective clothing/eye protection/ face protection.
- Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
- Use only outdoors or in a well-ventilated area.
- Store in a well-ventilated area.
- IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison center or doctor/physician if you feel unwell. Do not induce vomiting.
- IF SWALLOWED: call a poison center or doctor/physician if you feel unwell. Rinse mouth.
- IF ON SKIN: remove/ take off immediately all contaminated clothes, rinse skin with plenty of soap and water/ shower. Call a poison center or doctor/physician if you feel unwell.

- IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
- Wash contaminate clothing before reuse.

Sorption solution **SS**, Washing solution 1 **WB1**, Washing solution 2 **WB2** and Washing solution 3 **WB3** are highly flammable liquids.

Lysis buffer **LB** and Washing solution 1 **WB1** are harmful for aquatic life. Avoid release to the environment.

Precautionary statements according to Regulation EC № 1272/2008.

- To avoid the contamination:
  - use two separate pipette sets for DNA-free and DNA-containing components,
  - use filter tips,
  - do not use the same tip for two different components, neither DNA-free nor DNA-containing.
- Do not use the kit after its expiration date.
- Do not pool reagents from different lots or from different bottles of the same lot. Immediately after use, close all

bottles in order to avoid leakage. After opening, store all bottles and vials in an upright position.

- In case of using several Astra Biotech Infectious Real-Time PCR kits be sure of using **IC** from latest lot.

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