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REF K-1-1/B

IVD For *in Vitro* Diagnostic Use

CE

DNA-Sorb-B

DNA extraction kit

Key to symbols used

REF	List Number	2°C 25°C	Store at 2-25°C
IVD	For *in Vitro* Diagnostic Use		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
Σ	Contains reagents		Manufacturer

NAME

DNA-Sorb-B

INTENDED USE

The **DNA-Sorb-B** nucleic acid extraction kit is intended for the isolation and purification of DNA from plasma, serum, whole blood, liquor, amniotic liquid, tissue, urine, feces, bronco aspirates and other biological materials.

MATERIALS PROVIDED

- Lysis Solution, 15 ml;
- Washing Solution 1, 15 ml;
- Washing Solution 2, 50 ml;
- Sorbent, 1,25 ml;
- DNA-eluent, 5 ml.

Contains reagents for 50 tests.

MATERIALS REQUIRED BUT NOT PROVIDED

- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Tube racks
- Freezer
- Refrigerator

WARNINGS AND PRECAUTIONS

1. ⚠ Lysis Solution contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes in contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
2. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
3. Do not pipette by mouth.
4. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
5. Do not use a kit after its expiration date.
6. Dispose of all specimens and unused reagents in accordance with local regulations.
7. Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
8. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
9. 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.
10. Material Safety Data Sheets (MSDS) are available on request.
11. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
12. 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.

STORAGE AND SHIPPING

1. **DNA-Sorb-B** can be stored at 2-25°C storage temperature. Reagent will crystallize upon storage at 2-8°C.
2. **DNA-Sorb-B** can be shipped at room temperature.

STABILITY

DNA-Sorb-B is stable up to the expiration date indicated on the kit label.

SPECIMEN COLLECTION AND CONSERVATION

DNA-Sorb-B Kit can isolate DNA from:

- *whole blood* collected in either ACD or EDTA tubes;
- *serum* collected blood in Serum Separator tubes;
- *plasma* collected blood in ACD or EDTA tubes;
- *bone marrow aspirate* collected in EDTA tube;
- *liquor* stored in "Eppendorf" tube;
- *lacrimal liquid* stored in "Eppendorf" tube;
- *amniotic liquid* stored in "Eppendorf" tube;
- *sinovial liquid* stored in "Eppendorf" tube;
- *peritoneal and pleuric versament* stored in "Eppendorf" tube;
- *tissue* homogenized with mechanical homogenizer and dissolved in PBS sterile;
- *urine (sediment)*;
- *prostatic liquid* stored in "Eppendorf" tube;
- *seminal liquid:* transfer about 30 µl of seminal liquid to a polypropylene tube (1,5 ml) and add 70 µl of sterile saline solution;
- *sputum:* add 1 volume of sputum to 5 volumes of "Mucolisin" (reagent not provided). Vortex well and incubate at room temperature for 30 min, mix batchly. Transfer 1,0 ml of clinical material to a sterile 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;
- *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:*
  - ❖ *Bactery:*
    - o Prepare required quantity of 1,5 ml polypropylene tubes with 1,0 ml of Saline Solution. Add to each tube 0,1 g of feces. Vortex to get a homogeneous suspension and centrifuge for 5 min to 7000-12000g and using a micropipette with a plugged aerosol barrier tip transfer in a new tube 0,1 ml of the bacterial fraction (white-yellowish line between the sediment and the supernatant). Add 0,8 ml of sterile Saline Solution.
    - o Vortex vigorously and centrifuge for 5 min at 7000-12000g. Remove and discard the supernatant.
    - o Resuspend the pellet in 0,3 ml of Saline Solution
  - ❖ *Virus:*
    - o prepare required quantity of 1,5 ml polypropylene tubes with 1,0 ml of Saline Solution. Add to each tube 0,1 g of feces. Vortex vigorously to get the homogeneous suspension. Centrifuge for 5 min at 7-12000g and use the supernatant for the extraction of the DNA.

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.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60–65°C until disappearance of ice crystals. Prepare required quantity of 1.5 ml polypropylene tubes including one tube for **Negative Control of Extraction**.
2. Add to each tube **10 µl** of **Internal Control** (if provided with the amplification kit) and **300 µl** of **Lysis Solution**.
3. Add **100 µl** of **Samples** to the appropriate tube.
4. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Neg Control** provided with the amplification kit) to the tube labeled C*neg*.
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 7-10 sec. If the sample is not completely dissolved it is recommended to re-centrifuge the tube for 5 min at a maximum speed (12000-16000 g.) and transfer the supernatant into a new tube for DNA extraction.
6. Vortex vigorously **Sorbent** and add **20 µl** to each tube.
7. Vortex for 5-7 sec and incubate all tubes for 3 min at room temperature. Repeat this step.
8. Centrifuge all tubes for 30 sec at 5000g 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. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube.
10. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube.
11. Repeat step 10 and incubate all tubes with open cap for 5 min at 65°C.
12. Resuspend the pellet in **50 µl of DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
13. Centrifuge the tubes for 1 min at 12000g.
14. The supernatant contains 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.

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