REF K-1-8/100
VER 19.11.07

DNA-Sorb-D

DNA extraction kit

Key to symbols used

REF	List Number		Store at 2-8°C
RUO	For Research Use Only		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
	Contains reagents		Manufacturer

NAME
DNA-Sorb-D

INTENDED USE
The DNA-Sorb-D nucleic acid extraction kit is intended for the isolation and purification of DNA from liquid-based cytology samples (Cytoscreen, PreservCyt, etc).

MATERIALS PROVIDED
- Cytolisin, 2 x 5,0 ml;
- Mycolisin, 100 ml;
- PBS-buffer, 100 ml;
- Lysis Solution, 30 ml;
- Washing Solution, 100 ml;
- Sorbent, 3 x 1 ml;
- DNA-eluent, 2 x 5 ml.

Contains reagents for 100 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g);
- 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)
- 2,0 ml screw-cap tubes
- 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).

   Mycolisin contains mercaptoethanol. May be fatal if absorbed through skin. Harmful if swallowed or inhaled. Causes irritation to skin, eyes and respiratory tract. Combustible liquid and vapor. (R: 20/22-24-34-51/53; S: 26-36/37/39-45-61). Do not breathe vapor. Do not get in eyes, on skin, or on clothing. Keep container closed. Use with adequate ventilation. Wash thoroughly after handling. Keep away from heat and flame.
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-D** can be stored at 2-8°C. Reagent will crystallize upon storage at 2-8°C.
2. **DNA-Sorb-D** can be shipped at room temperature.

STABILITY

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

SPECIMEN COLLECTION, STORAGE AND TRANSPORT

DNA-Sorb-D Kit can isolate DNA from:

- Cervical swabs stored in liquid alcohol mediums for liquid-based cytology (Cytoscreen, PreservCyt, etc)

The DNA extraction from cells stored in this medium at room temperature can be performed also within several years.

SPECIMEN AND REAGENT PREPARATION

I step: Pretreatment of specimens

1. Prepare required quantity of 2,0 ml screw-cap tubes for the samples.
2. Vortex vigorously transport medium for liquid-based cytology and transfer 1,8 ml of cells to the appropriate tube.
3. Centrifuge the tubes at 12000g for 10 min. Discard the supernatant and leave about 150 µl of solution for DNA extraction.

II step: Cells washing

1. Add to the tubes **1 ml** of **Mycolisin**, vortex vigorously and incubate at room temperature for 30 min. Vortex periodically. Centrifuge the tubes at 12000g for 2 min. Discard the supernatant and leave about 150 µl of solution.
2. Add **1ml** of **PBS-buffer** to each tube. Vortex and centrifuge the tubes at 12000g for 2 min. Discard the supernatant without disturbing the pellet.
3. Vortex **Cytolisin**, add **0,1ml** to each tube and mix by pipetting. Vortex the tubes and incubate 2 hours (or overnight) at room temperature.

III step: DNA purification

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 one 1,5 ml tube for the Negative extraction Control.
2. Add to each tube **10 µl** of **Internal Control** (if provided with the amplification kit) and **300 µl** of **Lysis Solution**.
3. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Negative Control** provided with the amplification kit) to the tube labeled C*neg*.
4. 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.
5. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
6. Vortex for 5-7 sec and incubate all tubes for 3 min at room temperature. Repeat this step.
7. 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.
8. Add **500 µl** of **Washing Solution** to each tube. Vortex vigorously and centrifuge for 30 sec at 10000g. Remove and discard supernatant from each tube.
9. Repeat step 8 and incubate all tubes with open cap for 5-10 min at 65°C.
10. Resuspend the pellet in **100 µl of DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
11. Centrifuge the tubes for 1 min at 12000g.
12. 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.

CERMET SISTEMA DI GESTIONE CERTIFICATO 9001 : 2008

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CERMET SISTEMA DI GESTIONE CERTIFICATO 13485 : 2012