Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

DNA-Sorb-C

Nucleic acid extraction kit for the extraction and purification of DNA from clinical materials

USER MANUAL

REF **K-1-6/50**

Σ **50**

NAME

DNA-Sorb-C

INTENDED USE

The **DNA-Sorb-C** nucleic acid extraction kit is intended for the isolation and purification of DNA from tissue, animal feeds, pet foods.

PRINCIPLE OF ASSAY

DNA-sorb-C nucleic acid extraction kit is a reagent kit for rapid and efficient manual extraction and purification of DNA from various biological materials. Lysis Reagent Buffer and Washing Solution 1 contain chaotropic agents (guanidine chloride and guanidine thiocyanate), which lyse cells and denature cell proteins, respectively. The nucleic acids are then sorbed on silica particles. DNA extracted from biological samples may be used for PCR diagnostic tests.

MATERIALS PROVIDED

- **Lysis solution Buffer**, 20 ml;
- **Lysis reagent**, 0,85 ml;
- **Washing Solution 1**, 15 ml;
- **Washing Solution 2**, 50 ml;
- **Sorbent**, 1,25 ml;
- **DNA-eluent**, 5,0 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.

SPECIMEN COLLECTION AND CONSERVATION

DNA-Sorb-C kit can isolate DNA from:

- *tissue* homogenized with mechanical homogenizer and dissolved in PBS sterile;
- animal and poultry feed, pet food: take 5-10 gr of the sample, homogenise/crush in a clean mortar and dissolved in PBS sterile

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.

STORAGE AND SHIPPING

DNA-Sorb-C can be stored at 2-25°C storage temperature. Reagent will crystallize upon storage at 2-8°C.

DNA-Sorb-C can be shipped at room temperature.

STABILITY

DNA-Sorb-C 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 nucleic acid extraction kit are 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.

SPECIMEN AND REAGENT PREPARATION

1. **Lysis Solution Buffer** and **Washing Solution 1** and **2** (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 **17 µl** of **Lysis reagent** and **400 µl** of **Lysis Solution Buffer**.
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 60 min at 65°C. Vortex periodically.
6. Centrifuge the tubes for 5 min at a maximum speed (12000-14000 g.) and transfer the supernatant (200-350 µl) into a new tube for DNA extraction.
7. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
8. Vortex for 5-7 sec and incubate all tubes for 5 min at room temperature. Repeat this step.
9. Centrifuge all tubes for 60 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.
10. Add **300 µl** of **Washing Solution 1** to each tube. Vortex very vigorously and centrifuge for 60 sec at 5000g. Remove and discard supernatant from each tube.
11. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 60 sec at 10000g. Remove and discard supernatant from each tube
12. Repeat step 11 and incubate all tubes with open cap for 5-8 min at 65°C.
13. Resuspend the pellet in **50 µl of DNA-eluent.** Incubate for 5-8 min at 65°C and vortex periodically.
14. Centrifuge the tubes for 1 min at 12000g.
15. The supernatant contains DNA ready for amplification. If amplification is not performed in 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

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

REF	List Number		Caution!
LOT	Lot Number	Σ	Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at		Expiration Date
	Manufacturer		Warning
	Consult instructions for use		

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
*mail: **info@sacace.com** web: **www.sacace.com***