CE

IVD

For Professional Use Only

EDEM

Reagents kit for extraction of DNA by express method

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

Reagents kit for Extraction of DNA by Express Method (EDEM) is intended for the treatment of different types of clinical materials (urogenital swabs, throat swabs, conjunctiva swabs, erosive and ulcerative elements of mucous membranes and skin and first portions of human urine samples) with subsequent tests for the presence of STIs and other reproductive tract infections by using hybridization-fluorescence detection and PCR kits manufactured by FBIS CRIE.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

Clinical material, obtained from patient, is transferred into transport medium TM-EDEM, in such condition it is stored and transported to laboratory. For DNA extraction, a clinical sample aliquot is transferred into a tube with “IC-diluent”, then it is treated thermally with destruction of cell membranes, viral coats and other biopolymer complexes and DNA release. Insoluble components are pelleted on the tube bottom by centrifuging; the supernatant with DNA is used for PCR. The internal control sample (IC) contained in “IC-diluent” is isolated simultaneously with DNA from clinical material and, thereby, is a quality marker of laboratory analysis of clinical samples.

3. CONTENT

EDEM reagents kit is produced in 1 form:

EDEM reagents kit **REF** K2-17-100-CE

EDEM reagents kit includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
Transport MediumTM-EDEM	colorless, clear liquid	0.5	100 tubes
IC-diluent	colorless, clear liquid	0.3	100 tubes
PCR-buffer-Background	colorless, clear liquid	0.5	2 tubes

EDEM reagents kit is intended for DNA isolation from 100 samples of urogenital swabs, throat swabs, conjunctiva swabs, erosive and ulcerative elements of mucous membranes and skin, including controls. For DNA isolation from human urine samples, it is necessary to use an additional reagent, Transport Medium TM-EDEM (50 ml).

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat.
- Automated pipettors (dosers) of variable volumes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol barriers (up to 100 µl) in tube racks.

- Tube racks.
- Desktop microcentrifuge (till 16000 rpm)
- Vortex mixer/desktop centrifuge.
- Biological cabinet.
- Refrigerator for temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16°C.
- Waste bin for used tips.
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25°C and 100 °C.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- 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.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

Clinical material (except urine) is collected into tubes with Transport Medium TM-EDEM from EDEM reagents kit only.

Urogenital swabs, throat swabs, conjunctiva swabs, mucosa and skin erosive and ulcerative elements obtaining.

1. Open tubes with **TM-EDEM** after shaking off the drops of liquid from tube walls and cap to the bottom of the tube.
2. Place the working part of the probe with the clinical material into the transport medium TM-EDEM, break the shaft at the score line (if is present) and leave in the tube. If the score line is absent, place the working part of the probe into the medium, rotate it during 5-10 s pressing to the inside tube wall, and then remove the probe. Tightly close the tube.

Clinical material in the transport medium TM-EDEM in a tightly closed tube can be transported and stored:

–at room temperature (between 18 and 25 °C) for not more than 48 hours;

–at the temperature between 2 and 8°C for not more than 14 days;

–for longer storage, samples should be frozen at the temperature minus 20°C or below.

Obtaining the first urine portion

The first morning urine portion (15-25 ml) is collected into a special dry sterile 50 ml-bottle.

Urine samples can be transported and stored:

–at room temperature for 6 hours;

–at the temperature between 2 and 8 °C for 1 day.

Clinical material samples pretreatment (for urine only)

1. Shake the bottle with urine.
2. Add **1 ml** of urine into a 0.5 ml-tube with the transport medium TM-EDEM using a new pipette tip with aerosol barrier for each sample.
3. Centrifuge the tubes with TM-EDEM and urine at **12 000 rpm** for **5 min** to obtain pellet.
4. Not disturbing the pellet, remove the supernatant into a flask with a vacuum aspirator

using a new pipette tip without aerosol barrier for each sample.

5. Add **0.5 ml of TM-EDEM** into each tube with urine pellet using a new pipette tip with aerosol barrier for each sample. Tightly close the tubes, carefully vortex the content to resuspend the pellet, and precipitate the drops from tube walls and caps by short centrifuging for 2-3 s at 1.5 – 3 000 rpm.
6. Thus obtained samples in the transport medium TM-EDEM can be used for DNA isolation procedure as described above.

Obtained samples in the transport medium TM-EDEM can be stored:

–at room temperature (between 18 and 25 °C) for not more than 48 hours;

–at the temperature between 2 and 8°C for not more than 14 days;

–for longer storage, samples should be frozen at the temperature minus 20°C and below.

7. WORKING CONDITIONS

EDEM reagents kit should be used at 18–25 °C.

8. PROTOCOL

1. Switch on the thermostat and set the temperature at 95 °C.
2. Prepare and place the required number of tubes with **IC-diluent** into the tube rack and mark them. Precipitate the drops of solution from tube walls and caps by short centrifuging for 2-3 s at 1.5 – 3 000 rpm.
3. Before starting DNA isolation, mix the content of tubes with clinical material in transport medium TM-EDEM by vortexing and precipitate the drops of material from tube walls and caps by short centrifuging for 2-3 s at 1.5 – 3 000 rpm. Place the prepared tubes into tube rack.
4. Transfer **100 µl** of clinical material in the transport medium TM-EDEM into the prepared tubes with **IC-diluent** using a new pipette tip with aerosol barrier for each sample. Add **100 µl** of the transport medium TM-EDEM into the tube for Negative Control of Extraction (C-).
5. Tightly close all tubes, carefully, avoiding spraying, mix the content by vortexing, and place into the thermostat at **95 °C** for **5 min.**

If the tubes are not closed tightly, they can open during heating.

6. After the end of incubation, place the tubes into desktop centrifuge and centrifuge **for 1 min at 14 000 rpm.** Thus obtained DNA samples are ready for PCR analysis with hybridization-fluorescence detection.

DNA samples can be stored for one week at the temperature between 2 and 8°C or

for one year at the temperature not more than minus 16 °C (it is necessary to vortex and recentrifuge the tube content according to item 6 if PCR analysis of DNA samples is performed repeatedly).

For PCR analysis of obtained DNA samples by using PCR kit variant FEP, **PCR-buffer-Background** from reagents kit EDEM should be used for **Background** preparation. The tube **Background** is prepared as follows way: add 10 µl of **PCR-buffer-Background** into the tube with PCR-mix-1 on wax layer then add 10 µl of Negative Control of Extraction (C-) treated according to this instruction manual.

In case of invalid or equivocal result of PCR analysis obtained with the use of reagents kit EDEM it is necessary to repeat DNA isolation procedure. For it 100 µl of clinical material in transport medium TM-EDEM should be treated by using “DNA-sorb-AM” reagents kit according to its instruction manual.

If you have any questions or if you encounter problems, please contact our Authorized representative in the European Community.

9. TRANSPORTATION

EDEM reagents kit should be transported at 18–25 °C for no longer than 5 days.

10. STABILITY AND STORAGE

All components of the EDEM reagents kit are to be stored at the temperature between 2 ºC and 8 ºC when not in use. All components of the EDEM reagents kit are stable until the labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Transport medium TM-EDEM can be stored for no more than 14 days at the temperature below 25 C

11. REFERENCES

1. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2008.

12. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485 – certified Quality Management System, each lot of EDEM reagents kit has been tested against predetermined specifications to ensure consistent product quality.

13. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**IC**	Internal control
	Manufacturer	**C-**	Negative control of extraction
	Date of manufacture	**FBIS CRIE**	Federal Budget Institute of Science “Central Research Institute for Epidemiology”
EC REP	Authorised representative in the European Community		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.07.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”