EDEM

Reagents kit for extraction of DNA by express method

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit	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	Authorized representative in the European Community		

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.

Indications and contra-indications for use of the reagent kit

DNA extraction is used in preanalytical stage of in vitro diagnostics by nucleic acid amplification techniques (NAT).

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 extracted 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 K11-1581-100-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 extraction from 100 samples of urogenital swabs, throat swabs, conjunctiva swabs, erosive and ulcerative elements of mucous membranes and skin, including controls. For DNA extraction from human urine samples, it is necessary to use an additional reagent, **Transport Medium TM-EDEM** (50 ml).

4. ADDITIONAL REQUIREMENTS

- Pipettes (adjustable).
- Disposable tips with aerosol filters (up to 100 µl).
- Tube racks.
- Desktop microcentrifuge (RPM max. 16,000)
- Vortex mixer.
- PCR box or Biological cabinet.
- Thermostat for tubes with controlled temperature for 25-100 °C.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and 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 specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite, or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one directional, beginning in the Extraction Area and moving 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

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

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

Sampling

Urogenital swabs, oropharyngeal swabs, conjunctiva swabs, erosive-ulcerative lesions of mucous membrane and skin.

1. Open tubes with **Transport medium 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 **Transport medium TM-EDEM**, break off the shaft at the score line (if is present) and leave it 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 a tightly closed tube with **Transport medium TM-EDEM** can be transported and stored:

— at room temperature (18-25 °C) for not more than 48 hours;
— at 2-8°C for not more than 14 days;
— for longer storage, samples should be frozen at the temperature below minus 20°C.

Urine samples

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

Urine samples can be transported and stored:

— at room temperature for 6 hours;
— at 2-8°C for 1 day.

***Pretreatment* (for urine samples only)**

1. Shake the bottle with urine.
2. Add **1 ml** of urine into a 0.5 ml-tube with **Transport medium TM-EDEM** using a new pipette tip with aerosol filter for each sample.
3. Centrifuge the tubes with **Transport medium TM-EDEM** and urine at **12 000 rpm** for **5 min** to obtain pellet.
4. Without disturbing the pellet, remove the supernatant into a flask with a vacuum aspirator using a new pipette tip without aerosol filter for each sample.
5. Add **0.5 ml of Transport medium TM-EDEM** into each tube with urine pellet using a new pipette tip with aerosol filter 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 **Transport medium TM-EDEM** can be used for DNA extraction procedure as described above.

Obtained samples in **Transport medium TM-EDEM** can be stored:

— at room temperature (18-25 °C) for not more than 48 hours;
— at 2-8 °C for not more than 14 days;
— for longer storage, samples should be frozen at the temperature below minus 20°C.

Interfering substances and limitations of using test material samples

The information about potential interfering substances and limitations of using test material samples is specified in the Instruction Manual of the PCR kit.

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 extraction, 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 **Transport medium TM-EDEM** into the prepared tubes with **IC-diluent** using a new pipette tip with aerosol filter for each sample. Add **100 µl** of **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.**

NOTE: 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 2-8°C or for one year at the temperature from minus 24 to minus 16 °C (it is necessary to vortex and re-centrifuge the tube content according to item 6 if PCR analysis of DNA samples is performed repeatedly).

NOTE: For PCR analysis of obtained DNA samples by using PCR kit variant FEP, **PCR-buffer-Background** from **EDEM** reagents kit should be used for **Background** preparation. The tube **Background** is prepared as follows: 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.

NOTE: In case of invalid or equivocal result of PCR analysis obtained with the use of **EDEM** reagents kit it is necessary to repeat DNA extraction. 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 further questions or if you encounter problems, please contact our Authorized representative in the European Community.

9. TRANSPORTATION

EDEM reagents kit should be transported at 2–25 °C for no longer than 7 days.

10. STABILITY AND STORAGE

All components of **EDEM** reagents kit are to be stored at 2–8 °C when not in use. All components of **EDEM** reagents kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: 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, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.

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.

Please contact our Authorized representative in the European Community if side effects, undesirable reactions, facts and circumstances that pose a threat to the life and health of citizens and medical workers are identified during the use of the reagent kit.

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”
25.05.20 PM	3.Content, footer	REF K11-1581-100-CE was added
	Through the text	Corrections according to the template
21.10.20 MM	Footer, 3. Content	The information about REF K2-17-100-CE was deleted
11.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
31.05.22 KK	1. Intended use	“Indications and contra-indications for use of the reagent kit” subsection was added
	6. Sampling and handling	“Interfering substances and limitations of using test material samples” subsection was added
	13. Quality control	The Authorized representative in the European Community was specified for the contact in case of undesirable effects when using the reagent kit

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