



For *in Vitro* Diagnostic Use

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AmpliSens® EHEC-FRT

PCR kit

Instruction Manual



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1. INTENDED USE

AmpliSens® EHEC-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of enterohemorrhagic *E. coli* (*EHEC*) DNA in foods by using real-time hybridization-fluorescence detection.



The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

AmpliSens® EHEC-FRT PCR kit is intended for amplification of DNA of the genes encoding Shiga toxins 1 and 2 (Stx1/2) in *EHEC* and *Shigella* spp. Both microorganisms can cause diseases complicated by the hemorrhagic colitis and the hemolytic-uremic syndrome.

Enterohemorrhagic *E. coli* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® EHEC-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® EHEC-FRT** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® EHEC-FRT PCR kit is produced in 1 form:

AmpliSens® EHEC-FRT PCR kit variant FRT-50 F, **REF** R-B59(RG,iQ)-CE.

AmpliSens® EHEC-FRT PCR kit variant FRT-50 F includes:

| Reagent | Description | Volume (ml) | Amount |
|--------------------------------|------------------------|-------------|--------|
| PCR-mix-1-FL <i>EHEC</i> / STI | colorless clear liquid | 0.6 | 1 tube |
| PCR-mix-2-FRT | colorless clear liquid | 0.3 | 1 tube |
| Polymerase (TaqF) | colorless clear liquid | 0.03 | 1 tube |

| | | | |
|-----------------------------------------------------------------------------------------|------------------------|-----|--------|
| Positive Control DNA <i>E. coli</i> O:157 H:7 / STI (C+ _{<i>E. coli</i>} / sm) | colorless clear liquid | 0.1 | 1 tube |
| DNA-buffer | colorless clear liquid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless clear liquid | 1.2 | 1 tube |
| Internal Control-FL (IC)** | colorless clear liquid | 1.0 | 1 tube |

* must be used in the extraction procedure as Negative Control of Extraction (if the condition described in paragraph 3 of the Troubleshooting section is applied).

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see the DNA-sorb-B **REF** K1-2-50-CE and RIBO-prep **REF** K2-9-Et-50-CE protocols).

AmpliSens® EHEC-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia), iCycler iQ or iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

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 another 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 and then move to the Amplification and Detection Areas. 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® EHEC-FRT PCR kit is intended to analyze DNA extracted with DNA extraction kits from samples of primary enrichment media (selective liquid media used for detection of enterohemorrhagic *E.coli*, such as Kessler's medium, trypticase soy broth, brilliant green bile broth, or Gram-negative broth (GN broth)) prepared in accordance with effective regulatory documents.

7. PROTOCOL

7.1. DNA extraction

It's recommended that the following nucleic acid extraction kits are used:

- DNA-sorb-B **REF** K1-2-50-CE;
- RIBO-prep **REF** K2-9-Et-50-CE;
- Other nucleic acid extraction kits recommended by CRIE.



Extract DNA according to the manufacturer's instructions.



Use a sterile sample of the primary enrichment medium as a Negative Control of extraction (C-). It is recommended to examine a sterile sample of the primary enrichment medium for possible contamination with specific DNA before use.

To evaluate the viability of the studied microorganism, it is recommended to analyze two samples of the medium simultaneously. One sample should be collected after required incubation at a temperature optimal for the microorganism growth. The other one should be collected just after inoculation and kept at ≤ -20 °C before the study. If the signal of the sample that was kept at ≤ -20 °C lags behind 3 or more Ct units in the JOE/Yellow/HEX channel, it indicates the presence of a viable microorganism in the product.

7.2. Preparing PCR

7.2.1. Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

Mix the reaction mixture components just before use. Prepare the reaction mixture per required number of reactions (including clinical and controls samples) as specified in the Appendix 1. Carry out all control amplification reactions (positive (C+*E.coli* / STI) and negative (NCA)) for testing even one clinical or control sample. Prepare the reagent mixture for even number of reactions to attain more precise dispensing.

1. Thaw the reagents, vortex the tubes thoroughly, and make sure there are no drops on the walls of the tubes.
2. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
3. To prepare the reaction mixture mix the **PCR-mix-1-FL EHEC / STI**, **PCR-mix-2 FRT**, and **polymerase (TaqF)** according to Appendix 1. Vortex the tubes thoroughly. Make sure there are no drops on the walls of the tubes.
4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose unused reaction mixture.
5. Add **10 µl** of **DNA** obtained from clinical or control samples at the extraction stage into the prepared tubes using tips with aerosol barrier.



Avoid transferring sorbent together with the DNA sample in case of extraction by DNA-sorb-B kit.

6. Carry out the control amplification reactions:

NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+*E.coli* / STI -Add **10 µl** of **Positive Control DNA *E.coli* O:157 H:7 / STI** (to the tube labeled C+*E.coli* / STI (Positive Control of Amplification))

7.3.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual.

1. Create a temperature profile on your instrument as follows:

Table 1

Amplification program

| Step | Rotor-type Instruments ¹ | | | Plate-type Instruments ² | | |
|---------|-------------------------------------|---------------------------------------------|---------|-------------------------------------|---------------------------------------------|---------|
| | Temperature, °C | Time | Repeats | Temperature, °C | Time | Repeats |
| Hold | 95 | 15 min | 1 | 95 | 15 min | 1 |
| Cycling | 95 | 10 s | 45 | 95 | 10 s | 45 |
| | 60 | 25 s <i>fluorescent signal detection</i> | | 60 | 25 s <i>fluorescent signal detection</i> | |
| | 72 | 10 s | | 72 | 10 s | |

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step of stage Cycling.

- Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

8. DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

- The signal from the Internal Control DNA amplification product is detected in the FAM channel;
- The signal from the *EHEC* DNA amplification product is detected in the JOE/Yellow/HEX channel.

Results interpretation

The results are interpreted by the software of instrument by the crossing (or not-crossing) of the fluorescence curve with a threshold line and showed as presence (or absence) of Ct (threshold cycle) in the result grid.

Results should be interpreted in accordance with Table 2, Important Product Information Bulletin, and Guidelines.

¹ Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

² iCycler, iQ5, Mx3000P, Mx3000, DT-96, or equivalent.

Table 2

Result interpretation

| Ct value in the channel | | Result |
|--------------------------------------------|----------------------------------|----------------------------------------------------|
| FAM/Green | JOE/Yellow/HEX | |
| > boundary value or < boundary value | < boundary value | <i>EHEC</i> DNA is detected |
| < boundary value | absent or > boundary value | <i>EHEC</i> DNA is not detected |
| absent or > boundary value | absent or > boundary value | Invalid result Repeat extraction and PCR |

* For boundary values, see the *Important product information bulletin*.

Result of the analysis is considered reliable only if the results for both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 3).

Table 3

Results for controls

| Control | Stage for control | Ct value in the channel | |
|-----------------------------------------------|-------------------|----------------------------------|----------------------------------|
| | | FAM/Green | JOE/Yellow/HEX |
| C- | DNA extraction | ≤ boundary value | absent or > boundary value |
| NCA | PCR | absent or > boundary value | absent or > boundary value |
| C ⁺ _{<i>E.coli</i> / ST1} | PCR | < boundary value | < boundary value |

9. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If Ct value of the Positive Controls of PCR (C⁺_{*E.coli* / ST1}) is greater than boundary value in the JOE/Yellow/HEX channel the PCR and detection should be repeated for all samples in which *EHEC* DNA was not detected.
- If Ct value of the Negative Control of extraction (C-) and/or Negative Control of amplification (NCA) JOE/Yellow/HEX channel is less than boundary value, analysis should be repeated (starting from DNA extraction) for all samples in which *EHEC* DNA was detected.
- Positive result obtained for Negative Control of extraction (C-), that is a sterile sample of a culture medium, can indicate contamination of the primary enrichment medium with the genetic material of the examined microorganism. In this case the analysis should be repeated.

To do this, start from food primary enrichment with non-contaminated media and perform additional negative control extraction reaction using Negative Control reagent (see section 3. Content).

10. STABILITY AND STORAGE

All components of the **AmpliSens® EHEC-FRT** PCR kit are to be stored at 2–8 °C (except for PCR-mix-1-FL *EHEC* / STI, PCR-mix-2-FRT, and polymerase (TaqF)) when not in use. All components of the **AmpliSens® EHEC-FRT** PCR kit are stable until the expiration date on the label.



PCR-mix-1-FL *EHEC* / STI, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at ≤ –16 °C



PCR-mix-1-FL *EHEC* / STI is to be kept away from light

11. SPECIFICATIONS

11.1. Sensitivity

The analytical sensitivity of **AmpliSens® EHEC-FRT** PCR kit is specified in the table below.

| Clinical material | DNA extraction kit | PCR kit | Analytical sensitivity, GE/ml* |
|-------------------|--------------------|--------------------------|--------------------------------|
| Kessler's medium | DNA-sorb-B | PCR kit variant FRT-50 F | 1 x 10 ³ |
| Kessler's medium | RIBO-prep | PCR kit variant FRT-50 F | 1 x 10 ³ |

* Genome equivalents (GE) of the microorganism per 1 ml of a sample.

11.2. Specificity

The analytical specificity of **AmpliSens® EHEC-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. There were no nonspecific test responses during examination of human DNA as well as a DNA panel of the below microorganisms:

- 31 strains of different serogroups of *Esherichia coli* (including *EHEC*, *EPEC*, *ETEC*, *EAggEC*, *EIEC*), 3 strains of *Cronobacter sakazakii*, 4 strains of *Enterobacter cloacae*, 2 strains of *Enterobacter aerogenes*, 2 strains of *Pantoea agglomerans*, 8 strains of *Campylobacter* spp. (*C.jejuni*, *C.coli*, and *C.fetus fetus*), 18 strains of different serogroups of *Salmonella* spp., 12 strains of different species and serogroups of *Shigella* spp., 22 strains of different species and serogroups of *Yersinia* spp., *Citrobacter freundii*, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Protrus mirabilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*.

The clinical specificity of **AmpliSens® EHEC-FRT** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal State Institution of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL

In accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® EHEC-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Caution, consult accompanying documents



Contains sufficient for <n> tests



Internal Control



Consult instructions for use



Negative control

C⁺*E.coli* / STI

Positive Control DNA *E.coli* O:157 H:7 / STI

NCA

Negative Control of Amplification

CRIE

Central Research Institute of Epidemiology, Moscow, Russia

ETEC

Enterotoxigenic *E.coli*

EPEC

Enteropathogenic *E.coli*

EHEC

Enterohemorrhagic *E.coli*

EIEC

Enteroinvasive *E.coli*

EAgEC

Enterocytotoxic *E.coli*

List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|----------|---------------------|-----------------------------------------------------------------------------------------------------------------|
| 02.11.10 | Through the text | Abbreviation C ⁺ <i>E.coli</i> / STI is added for Positive Control DNA <i>E.coli</i> O:157 H:7 / STI |