



For Professional Use Only

# eSens Rotavirus/Norovirus/Astrovirus QL PCR kit

**REF** ES3540B

## Instructions for Use

### 1 INTENDED USE

**eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *rotavirus A*, *norovirus* genotype 2, and *astrovirus* RNA in the environmental samples (water sample concentrates) and clinical material (feces) using real-time hybridization-fluorescence detection of amplified products.

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

### 2 PRINCIPLE OF PCR DETECTION

Detection of *rotavirus A*, *norovirus* genotype 2, and *astrovirus* RNA includes RNA extraction from test samples and reverse transcription of RNA into cDNA combined with real-time PCR amplification of cDNA (RT-PCR). In the real-time PCR, the amplified product is detected with the use of 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.

**eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is a qualitative test that contains the Internal Control (Internal Control-FL (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.

**eSens Rotavirus/Norovirus/Astrovirus QL 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 a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
<b>Name of PCR-mix</b>	<b>DNA-target</b>	
RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	<i>Rotavirus</i> grA RNA	<i>Astrovirus</i> RNA
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	Internal Control cDNA	<i>Norovirus</i> G2 RNA
<b>Name of PCR-mix</b>	<b>Target gene</b>	
RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	NSP2	Gene for capsid protein
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	Artificially synthesized sequence	Gene for capsid protein

### 3 CONTENT

#### eSens Rotavirus/Norovirus/Astrovirus QL PCR kit (ES3540B)

Reagent	Description	Volume, ml	Quantity
<b>RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i></b>	clear liquid from colorless to light lilac colour	0.6	1 tube
<b>RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i></b>	clear liquid from colorless to light lilac colour	0.6	1 tube
<b>PCR-buffer-C</b>	colorless clear liquid	0.3	2 tubes
<b>Polymerase (TaqF)</b>	colorless clear liquid	0.03	2 tubes
<b>TM-Revertase (MMIv)</b>	colorless clear liquid	0.015	2 tubes
<b>RT-G-mix-2</b>	colorless clear liquid	0.015	2 tubes
<b>Positive Control cDNA <i>Rotavirus-Flu / Astrovirus</i> (C<sup>+</sup> <i>Rotavirus / Astrovirus</i>)</b>	colorless clear liquid	0.1	1 tube
<b>Positive Control cDNA <i>Norovirus</i> genotype 2-Flu /STI (C<sup>+</sup> <i>Norovirus</i> genotype 2 /STI)</b>	colorless clear liquid	0.1	1 tube
<b>TE-buffer</b>	colorless clear liquid	0.5	1 tube
<b>Negative Control (C-)*</b>	colorless clear liquid	1.6	1 tube
<b>Internal Control-FL (IC)**</b>	colorless clear liquid	0.12	5 tubes
<b>Buffer for elution B***</b>	colorless clear liquid	1.2	5 tubes

\* must be used in the extraction procedure as Negative Control of Extraction

\*\* add **10 µl** of **Internal Control-FL (IC)** during the RNA extraction procedure directly to the sample/lysis mixture

\*\*\* must be used in the extraction procedure.

**eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is intended for 55 reactions (including controls).

## 4 ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real-time instruments for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes:
  - a) 0.2-ml tube (flat cap, nonstriped) for 36-well rotor if a rotor-type instrument is used.
  - b) 0.2-ml tube (domed cap) if plate-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

## 5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a 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 afterwards.
- 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 samples or reagents 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 in the area where 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

**eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is intended for analysis of the RNA extracted with RNA extraction kits from:

- 6.1 *Water sample concentrates* (pretreatment is not required).
- 6.2 *Feces* (pretreatment should be carried out).

## 7 WORKING CONDITIONS

**eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

## 8 PROTOCOL

### 8.1 RNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

#### **Ecoli Dx, s.r.o. recommends:**

- For the manual extraction
  - **RIBO-prep** (K2-9-Et-100-CE)
- For the automatic extraction
  - **ePure Viral Nucleic Acid Extraction Kit** (E2003)

**NOTE:** Extract the RNA according to the manufacturer's protocol.  
The RNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC)**.  
Use Buffer for elution B included in this PCR kit during RNA extraction.

### 8.2 Preparing RT-PCR

#### 8.2.1 Preparing tubes for RT-PCR

The type of tubes depends on the type of PCR real-time instrument. Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

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

1. Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (including test and control samples) according to Table 2. Note that even for analysis of one test or control RNA sample it is necessary to carry out all controls of RT-PCR (positive (C+) and negative (NCA)) for each RT-PCR-mix-1. It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.
2. Take the required number of tubes for amplification of test and control samples.
3. To prepare the reaction mixture, mix one of the **RT-PCR-mix-1-FEP/FRT (RT-PCR-mix-1-FEP/FRT Rotavirus / Astrovirus** or **RT-PCR-mix-1-FEP/FRT Norovirus / STI)**, **PCR-buffer-C**, **polymerase (TaqF)**, and **TM-Revertase (MMLv)** according to Table 1. Vortex the tubes thoroughly. Make sure that there are no drops on the walls of the tubes.
4. Transfer **15 µl** of the prepared mixture to the prepared tubes. Dispose of the unused reaction mixture.

Table 2

## Scheme of reaction mixture preparation

Reagent volume per one reaction, $\mu\text{l}$		Reagent volume for specified number of reactions, $\mu\text{l}$				
		10.00	5.00	0.25	0.50	0.25
Number of test samples	Number of reactions*	RT-PCR-mix-1-FEP/FRT	PCR-buffer-C	RT-G-mix-2	Polymerase (TaqF)	TM-Revertase (MMIv)
2	6	60	30	1.5	3.0	1.5
4	8	80	40	2.0	4.0	2.0
6	10	100	50	2.5	5.0	2.5
8	12	120	60	3.0	6.0	3.0
10	14	140	70	3.5	7.0	3.5
12	16	160	80	4.0	8.0	4.0
14	18	180	90	4.5	9.0	4.5
16	20	200	100	5.0	10.0	5.0
18	22	220	110	5.5	11.0	5.5
20	24	240	120	6.0	12.0	6.0
22	26	260	130	6.5	13.0	6.5
24	28	280	140	7.0	14.0	7.0
26	30	300	150	7.5	15.0	7.5
28	32	320	160	8.0	16.0	8.0

\* Test samples (N) + control of RNA extraction + 2 controls of RT-PCR + extra reaction (N+1+2+1).

5. Add **10  $\mu\text{l}$**  of **RNA** obtained at the RNA extraction stage to the prepared tubes using tips with aerosol filter.

6. Carry out the control amplification reactions:

<b>NCA</b>	– Add <b>10 µl</b> of <b>TE-buffer</b> to the tube labeled NCA (Negative Control of Amplification).
<b>C+<sub>Rotavirus / Astrovirus</sub></b>	– Add <b>10 µl</b> of <b>Positive Control cDNA Rotavirus-Flu / Astrovirus (C+<sub>Rotavirus / Astrovirus</sub>)</b> (in case of using RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i> ) to the tube labeled C+ <sub>Rotavirus / Astrovirus</sub> (Positive Control of Amplification)
<b>C+<sub>Norovirus genotype 2 / STI</sub></b>	– Add <b>10 µl</b> of <b>Positive Control cDNA Norovirus genotype 2-Flu / STI (C+<sub>Norovirus genotype 2 / STI</sub>)</b> (in case of using RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i> ) to the tube labeled C+ <sub>Norovirus genotype 2 / STI</sub> (Positive Control of Amplification)
<b>C–</b>	– Add <b>10 µl</b> of <b>the sample extracted from the Negative Control (C–) reagent</b> to the tube labeled C– (Negative control of Extraction).

### 8.2.2 Amplification

1. Create a temperature profile on your instrument as follows **(Table 3,4)\***

#### eSens unified amplification program for rotor-type and plate-type instruments

**Table 3**

Step	Temperature, °C	Time	Cycles
<b>1</b>	<b>50</b>	<b>15 min</b>	<b>1</b>
<b>2</b>	<b>95</b>	<b>15 min</b>	<b>1</b>
<b>3</b>	<b>95</b>	<b>10 s</b>	<b>45</b>
	<b>60</b>	<b>20 s</b> <b>Fluorescence acquiring</b>	

**NOTE:** Fluorescent signal is detected in the channels for the **FAM** and **JOE** fluorophores.

Any combination of the test (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in „multiprime“ format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones.

\* The amplification programs **(Table 3,4)** are equivalent for the use with this PCR kit.

Table 4

### Amplification program for rotor-type and plate-type instruments

Step	Temperature, °C	Time	Cycles
1	50	30 min	1
2	95	15 min	1
3	95	10 s	45
	60	25 s Fluorescence acquiring	
	72	10 s	

Fluorescent signal is detected in the channels for the **FAM** and **JOE** fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

- Adjust the fluorescence channel sensitivity.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

### 8.3 Instrument Settings

#### Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 5FI to 10FI	0.05	On	On	5-10 %
JOE/Yellow	from 5FI to 10FI	0.05	On	On	10 %

#### Test settings for plate-type instruments

**Note:** Set **Ramp Rate 2,5 °C/s** by clicking the **Step Options** button for each step of cycling.

Channel	Threshold
FAM JOE/HEX	The threshold line for each channel is set at the level of 10-20% of maximum fluorescence obtained for the Positive Controls in the last amplification cycle.

## 9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels (see table 5).

Table 5

#### Correspondence table of detection channels, RT-PCR-mixes-1 and pathogens

Channel for fluorophore	RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>
FAM	<i>Rotavirus</i> grA cDNA	Internal Control-FL cDNA
JOE	<i>Astrovirus</i> cDNA	<i>Norovirus</i> G2 cDNA

## Result interpretation

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value of the RNA sample in the corresponding column of the results grid.

Results should be interpreted in accordance with Table 6.

**Table 6**

### Interpretation of results

Channel for fluorophore	RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>
FAM	< boundary value <i>Rotavirus</i> grA RNA is detected	< boundary value IC cDNA is detected. The result of sample is valid
	Absent or > boundary value <i>Rotavirus</i> grA RNA is not detected*	Absent or > boundary value Invalid result**
JOE	< boundary value <i>Astrovirus</i> RNA is detected	< boundary value <i>Norovirus</i> G2 RNA is detected
	Absent or > boundary value <i>Astrovirus</i> RNA is not detected*	Absent or > boundary value <i>Norovirus</i> G2 RNA is not detected*

\* Only if the Ct value for RT-PCR-mix-FEP/FRT *Norovirus / STI* in the FAM channel is less than the boundary value.

\*\* If Ct value for RT-PCR-mix-FEP/FRT *Norovirus / STI* in the FAM channel is absent or greater than the boundary value, the negative result obtained with the other PCR-mix-1 is considered invalid; therefore, the sample should be examined once again starting from RNA extraction.

**The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 7, Table 8 and Table 9).**

**Table 7**

### Results for controls

RT-PCR-mix-1	Control	Stage for control	Ct value in the channel for fluorophore	
			FAM	JOE
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	C <sup>-</sup>	RNA extraction	≤ boundary value	Absent or > boundary value
	NCA	PCR	Absent or > boundary value	Absent or > boundary value
	C <sup>+</sup> <i>Norovirus</i> genotype 2 / STI	PCR	< boundary value	< boundary value

RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	C-	RNA extraction	Absent or > boundary value	Absent or > boundary value
	NCA	PCR	Absent or > boundary value	Absent or > boundary value
	C+ <i>Rotavirus / Astrovirus</i>	PCR	< boundary value	< boundary value

**Table 8**

**Boundary Ct values for rotor-type instruments**

**Clinical samples**

RT-PCR-mix-1-FEP/FRT	Channel for fluorophore	
	FAM	JOE
RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	38	38
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	38	38

**Environmental samples**

RT-PCR-mix-1-FEP/FRT	Channel for fluorophore	
	FAM	JOE
RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	40	40
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	38	40

**Table 9**

**Boundary Ct values for plate-type instruments**

**All types of test material**

RT-PCR-mix-1-FEP/FRT	Channel for fluorophore	
	FAM	JOE
RT-PCR-mix-1-FEP/FRT <i>Rotavirus / Astrovirus</i>	40	40
RT-PCR-mix-1-FEP/FRT <i>Norovirus / STI</i>	40	40

## 10 TROUBLESHOOTING

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

1. If the Ct value determined for the Positive Control of amplification (C+) in the channels for the JOE and FAM fluorophores is greater than the boundary Ct value, the amplification and detection should be repeated for all samples in which the signal in the channels for the JOE and FAM fluorophores was greater than the boundary value with the appropriate RT-PCR-mix.
2. If the signal for the Negative control of extraction (C-) (except for RT-PCR-mix-1-FEP/FRT *Norovirus / STI*) and/or the Negative control of amplification (NCA) in the channels for the JOE and FAM fluorophores is less than the boundary value, PCR should be repeated (starting from RNA extraction stage) for all samples in which the pathogen cDNA was detected.

## 11 TRANSPORTATION

**eSens Rotavirus/Norovirus/Astrovirus QL PCR** kit should be transported at 2–8 °C for no longer than 5 days.

## 12 STABILITY AND STORAGE

All components of the **eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** are to be stored at 2–8 °C when not in use (except for RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus / STI*, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMIv), and RT-G-mix-2). All components of the **eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

**NOTE:** RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus*, RT-PCR-mix-1-FEP/FRT *Norovirus / STI*, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMIv), and RT-G-mix-2 are to be stored at temperature from minus 24 to minus 16 °C.

**NOTE:** RT-PCR-mix-1-FEP/FRT *Rotavirus / Astrovirus* and RT-PCR-mix-1-FEP/FRT *Norovirus / STI* are to be kept away from light

## 13 SPECIFICATIONS

### 13.1 Sensitivity

The analytical sensitivity of **eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is specified in the table below.

Pathogen	Test material	RNA/DNA extraction kit	Analytical sensitivity, GE/ml*
<i>Rotavirus A</i>	Feces	RIBO-prep	1 x 10 <sup>4</sup>
<i>Norovirus</i> genotype 2	Feces	RIBO-prep	5 x 10 <sup>3</sup>
<i>Astrovirus</i>	Feces	RIBO-prep	1 x 10 <sup>4</sup>

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

### 13.2 Specificity

The analytical specificity of **eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Specificity was confirmed on the following microorganism strains: *Enterovirus* strains (Coxsackie B1, B2, B3, B4, B5, and B6; Polio (Sabin) I, II, and III); *Adenovirus* serogroups 5 and 7; *influenza virus A* (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, and H5N1) and B; *rhinoviruses*; *RS viruses*; *human adenovirus* types 3, 5, 7, 37, and 40; *Salmonella enteritidis* S-6, *S.choleraesuis* 370, *S.typhimurium* 371, *S.dublin* 373, *S.typhi* C1, *S.abortusovis* 372, and *S.gallinarum-pullorum*;; *Shigella flexneri* 851b; *Campylobacter fetus* ssp. *fetus* 25936 and *C.jejuni* ssp. *jejuni* 43435; *Clebsiella* K 65 SW4; *Listeria monocitogenes* USKHCH 19 and *L.monocitogenes* USKHCH 52; *Proteus vulgaris* 115/98; *Pseudomonas aeruginosa* DN c1; *Staphilococcus aureus* 653 and *S. aureus* 29112; *Morganella morganii* 619 c 01; and *Enterobacter faecalis* 356; as well as 44 *Norovirus* isolates of different gene clusters of genotypes 1 and 2; 40 *Rotavirus* strains of different [P]G types, 19 *Astrovirus* strains of serogroups 1, 2, 4, 5, and 8; and 15 *Adenovirus* strains of different types and the following bacterial strains (see table 10).

**Table 10**

#### The panel of bacterial pathogens Center for Disease Control and Prevention (CDC, USA)

Strain ID	Organism	Strain ID	Organism
K2033	<i>Salmonella</i> ser. Grumpensis	K2015	<i>Salmonella</i> ser. Oranienburg
K1806	<i>Salmonella</i> ser. Newport	AM01144	<i>Salmonella</i> ser. Newport
K2077	<i>Salmonella</i> ser. Enteritidis	K1810	<i>Salmonella</i> ser. Anatum
83-99	<i>Salmonella</i> ser. Typhimurium	K1991	<i>Salmonella</i> ser. Typhimurium
PS505	<i>Shigella boydii</i>	K1898	<i>Salmonella</i> ser. Heidelberg
PS408	<i>Shigella sonnei</i>	PS555	<i>Shigella boydii</i>
B4003	<i>Shigella sonnei</i>	F6446	<i>Shigella dysenteriae</i>
PS801	<i>Shigella dysenteriae</i>	S821X1	<i>Shigella dysenteriae</i> type 1

C898	<i>Shigella dysenteriae</i> type1	S177X1	<i>Shigella dysenteriae</i> type 1
F2035	<i>Shigella flexneri</i>	S3314	<i>Shigella dysenteriae</i> type 2
E2539-C1	Enterotoxigenic <i>Escherichia coli</i> (ETEC)	PS071	<i>Shigella flexneri</i>
H10407	Enterotoxigenic <i>Escherichia coli</i> (ETEC)	PS050	<i>Shigella flexneri</i>
F1008	Enterotoxigenic <i>Escherichia coli</i> (ETEC)	F7862	<i>Shigella flexneri</i>
EDL 933	Shiga-toxin <i>E. coli</i> (STEC)	TX1	Enterotoxigenic <i>Escherichia coli</i> (ETEC)
3543-01	Shiga-toxin <i>E. coli</i> (STEC)	3525-01	Shiga-toxin <i>Escherichia coli</i> (STEC)
4752-71	<i>Proteus vulgaris</i>	25922	<i>Escherichia coli</i> O6:H1
QA/QC	<i>Citrobacter freundii</i>	621-64	<i>Citrobacter freundii</i>
QA/QC	<i>Aeromonas</i>	3910-68	<i>Aeromonas</i> spp.
3043-74	<i>Serratia marcescens</i>	E9113	<i>Vibrio cholerae</i>
QA/QC	<i>Serratia marcescens</i>	501-83	<i>Edwardsiella</i> spp.
F7894	<i>Vibrio vulnificus</i>	587-82	<i>Providencia stuartii</i>
F8515	<i>Yersinia enterocolitica</i>	27853	<i>Pseudomonas aeruginosa</i>
F8510	<i>Yersinia enterocolitica</i>	D4989	<i>Helicobacter cinaedi</i>
K4299	<i>Vibrio parahaemolyticus</i>	D6827	<i>Helicobacter pullorum</i>
F9835	<i>Vibrio parahaemolyticus</i>	D5127	<i>Helicobacter pylori</i>
K2023	<i>Salmonella</i> ser. Kentucky	D2686	<i>Arcobacter butzleri</i>
K1684	<i>Salmonella</i> O-1, 4, 12 gr. B		

There were no nonspecific test responses during examination of human DNA as well as a DNA panel of the above-mentioned microorganisms.

The clinical specificity **eSens Rotavirus/Norovirus/Astrovirus QL PCR kit** was confirmed in laboratory clinical trials.

## 14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

## 15 KEY TO SYMBOLS USED

 REF	Catalogue number		Caution
 LOT	Batch code		Contains sufficient for <n> tests
 IVD	<i>In vitro</i> diagnostic medical device		Use-by Date
 VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	<b>NCA</b>	Negative control of amplification
	Date of manufacture	<b>C+ Rotavirus</b> / <i>Astrovirus</i>	Negative control of extraction
	Authorized representative in the European Community	<b>C+ Norovirus</b> genotype 2 / STI	Positive control of amplification
<b>C-</b>	Negative control of extraction	<b>IC</b>	Internal control

### List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		
02_07/2025	Through the text	Corrections according to the Tables.
	3 CONTENT	Table was changed.
	7 WORKING CONDITIONS	Temperature range was changed.
	8 PROTOCOL	Table was changed.
	13 SPECIFICATIONS	Table was changed.

Ecoli Dx, s.r.o. , Purkyňova 74/2



110 00 Praha 1, Česká republika  
Tel: +420 325 209 912

Mobil: +420 739 802 523