ECOLI

eSens

CE

IVD

For Professional Use Only

eSens ARVI screen QL PCR kit

REF **ES3304A**

Instructions for Use

1 INTENDED USE

eSens ARVI screen QL PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection and identification of specific nucleic acid fragments of pathogens that cause **a**cute **r**espiratory **v**iral **i**nfections – *human Respiratory Syncytial virus* (*hRSv*) RNA; *human Metapneumovirus* (*hMpv*) RNA; *human Parainfluenza virus*-1-4 (*hPiv*) RNA; OC43, E229, NL63, and HKUI *human Coronavirus* (*hCov*) RNA; *human Rhinovirus* (*hRv*) RNA; *human* B, C, and E *Adenovirus* (*hAdv*) DNA; and *human Bocavirus* (*hBov*) DNA – in the clinical material (nasal and oropharyngeal swabs, sputum, aspirate of trachea, bronchoalveolar lavage, bronchial washing fluid, and autopsy material) by 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

ARVI detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific ARVI 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 PCR 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 ARVI screen QL PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-rec (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 ARVI screen 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 using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and

catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So, the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

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

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**
PCR-mix-1-FL-F	**cDNA-target**		
hRSv – hMpv	Internal Control STI-rec cDNA	*hRSv* cDNA	*hMpv* cDNA
hPiv 1/3		*hPiv* 3 cDNA	*hPiv* 1 cDNA
hPiv 2/4		*hPiv* 2 cDNA	*hPiv* 4 cDNA
hCov		*NL-63, 229E* cDNA	*HKU-1, OC 43* cDNA
hAdv – hBov		*hBov* cDNA	*hAdv* cDNA
hRv		—	*hRv* cDNA
PCR-mix-1-FL-F	**Target gene**		
hRSv – hMpv	Artificially synthesized sequence	Nucleoprotein N gene	Nucleoprotein N gene
hPiv 1/3		HN gene	HN gene
hPiv 2/4		HN gene	HN gene
hCov		Nucleocapsid protein (N) gene	Nucleocapsid protein (N) gene
hAdv – hBov		NP gene	Hexon gene
hRv		—	5’ UTR

3 CONTENT

eSens ARVI screen QL PCR kit (ES3304A) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL-F *hRSv – hMpv*	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-1-FL-F *hPiv* 1/3	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-1-FL-F *hPiv* 2/4	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-1-FL-F *hCov*	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-1-FL-F *hAdv – hBov*	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-1-FL-F *hRv*	clear liquid from colorless to light lilac colour	0.2	5 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	6 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	6 tubes
Positive Control cDNA *hRSv - hMpv* (C+$_{hRSv\text{-}hMpv}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA *hPiv* 1/3 (C+$_{hPiv\ 1/3}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA *hPiv* 2/4 (C+$_{hPiv\ 2/4}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA *hRv* (C+$_{hRv}$)	colorless clear liquid	0.1	2 tubes
Positive Control cDNA *hCov* (C+$_{hCov}$)	colorless clear liquid	0.1	2 tubes
Positive Control DNA *hAdv – hBov* (C+$_{hAdv\text{-}hBov}$)	colorless clear liquid	0.1	2 tubes
Positive Control STI-88 (CS+)	colorless clear liquid	0.1	6 tubes
TE-buffer	colorless clear liquid	0.5	2 tubes
Negative Control (C–)*	colorless clear liquid	1.2	2 tubes
Internal Control STI-rec (IC)**	colorless clear liquid	0.12	10 tubes

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

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

eSens ARVI screen QL PCR kit is intended for 100 reactions for each PCR-mix-1-FL-F (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Reverse transcription kit.
- Transport medium.
- 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 a 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 (0.1- or 0.2-ml)
    - 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
    - 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-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 ARVI screen QL PCR kit is intended for analysis of DNA/RNA extracted from the clinical material:

- nasal and oropharyngeal swabs;
- sputum (or aspirate of trachea or throat);
- bronchoalveolar lavage or bronchial washing fluid;
- autopsy material.

Sampling:

1. *Nasal swab samples* are obtained using sterile dry flocked swabs with plastic shafts for nasopharyngeal swabs. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Gently insert the swab along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall.

   When the material is obtained, insert the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (**REF** 959-CE, **REF** 957-CE, **REF** 958-CE). Break off the end of shaft to allow tight closing of the tube cap. Close the tube with the solution and the swab.

2. *Oropharyngeal swab samples* are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water.

   When material is obtained, insert the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (**REF** 959-CE, **REF** 957-CE, **REF** 958-CE). Break off the end of shaft to allow tight closing of tube cap. Close the tube with the solution and the swab.

   NOTE: It is recommended to combine nasal and oropharyngeal swabs in a single tube. For this purpose, place the ends of both shafts into one tube and analyze them as a single sample

3. Nasopharyngeal sputum or aspirate or tracheal sputum or aspirate

   Collect sputum into sterile disposable container after gargling the oral cavity with water. Collect nasopharyngeal or tracheal aspirate by the conventional procedure and transfer them into sterile disposable containers.

4. Bronchoalveolar lavage and bronchial washing fluid

   Collect bronchoalveolar lavage and bronchial washing fluid by the conventional procedure and transfer them into sterile disposable containers.

   Store the samples at 2–8 °C for 1 day or at not more than minus 16 °C for 1 week.

5. *Autopsy sample* should be immediately placed in a sterile disposable container and frozen otherwise it should be examined within 1 hour from the time of sample collection. Store the samples at minus 68 °C for 1 year.

   NOTE: Only one freeze-thaw cycle of clinical material is allowed.

Pretreatment

6. Nasal and oropharyngeal swabs.

   Vortex the tube, then centrifuge it at 5,000 rpm for 5 s to sediment drops from the interior wall of the tube lid.

7. Nasopharyngeal sputum or aspirate or tracheal sputum or aspirate.

   Use reagent **Mucolysin** (180-CE) sputum and aspirate pretreatment. See the instruction manual to **Mucolysin** for a proper use.

   The pretreated sputum (100 µl) is used for RNA/DNA extraction. If it is necessary to repeat the test, the rest of sputum can be frozen.

8. Bronchoalveolar lavage and bronchial washing fluid

   Use 100 µl of material sample for extraction. If it is necessary to repeat the test, the remaining material can be frozen.

9. *Autopsy material* is homogenized using sterile porcelain mortars and pestles. Then, prepare a 10 % suspension in a sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube and centrifuge at 10,000 rpm for 5 min. The supernatant (100 µl) is used for DNA/RNA extraction. If it is necessary to repeat the test, the remaining suspension can be frozen.

7 WORKING CONDITIONS

eSens ARVI screen QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA 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)

The DNA/RNA extraction from each clinical sample is carried out in the presence of **Internal Control STI-rec (IC)**.

In the extraction procedure for each panel it is necessary to carry out the control reaction as follows:

C–	Add **100 µl of Negative Control (C–)** to the tube labelled C–.

8.2 Reverse transcription

It is recommended to use following RT reagents kits for complementary DNA (cDNA) synthesis from RNA.

- **REVERTA-L** (K3-4-100-CE), which contains RT-G-mix-1 (2 kits required). The **Reverse transcription** procedure is described below.

Total reaction volume – **40 µl**, volume of RNA sample - **20 µl**.

1. Prepare required number of 0.2 (0.5) ml disposable polypropylene microcentrifuge tubes.
2. Prepare ready-to-use reagent mix for 6 reactions.
   a. Add **5 µl** of **RT-G-mix-1** to the tube containing **RT-mix**, carefully mix on vortex for 3 s, centrifuge for 5-7 s (for removing drops from the internal surface of the test tubes caps).
   b. Add **6 µl** of **Revertase (MMlv)** into the tube with reagent mix, then pipette 5 times and mix on vortex for 3 s, and then centrifuge for 5-7 s (to remove any drop adhering to the internal surface of the test tubes caps).
3. Dispense **20 µl** of ready-to-use reagent mix into each prepared test tube.
4. Add **20 µl RNA-sample** to the appropriate test tube with ready-to-use reagent mix. Carefully mix, using the pipette.
5. Place the test tubes into thermocycler and incubate at 37 °C for 30 minutes.
6. Dilute each cDNA sample in the ration 1:1 with DNA-buffer. To do that, add **40 µl** DNA-buffer to each test tube. Carefully mix, using the pipette (10 times).

cDNA samples can be stored at the temperature not more than minus 16 °C for a week or at the temperature not more than minus 68 °C for a year.

8.3 Preparing PCR

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

NOTE: At the amplification step, positive controls, CS+, and NCA are used in every experiment in order to control reagent purity and carefulness of operator's work. C– is also tested at the amplification step.

Table 2

Compliance of names of PCR-mixes-1-FL and positive controls of ARVI pathogens

PCR-mix-1-FL	Positive control (C+)
hRSv - hMpv	Positive Control cDNA *hRSv – hMpv* (C+$_{hRSv\text{-}hMpv}$)
hAdv - hBov	Positive Control DNA *hAdv – hBov* (C+$_{hAdv\text{-}hBov}$)
hRv	Positive Control cDNA *hRv* (C+$_{hRv}$)
hPiv 1/3	Positive Control cDNA *hPiv* 1/3 (C+$_{hPiv\ 1/3}$)
hPiv 2/4	Positive Control cDNA *hPiv* 2/4 (C+$_{hPiv\ 2/4}$)
hCov	Positive Control cDNA *hCov* (C+$_{hCov}$)

8.3.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Thaw the required number of tubes with the corresponding PCR-mix-1-FL. Vortex the tubes with **PCR-mix-1-FL-F, PCR-mix-2-FRT,** and **polymerase (TaqF)** and then centrifuge briefly.
2. Take the required number of tubes/strips for amplification of the cDNA obtained from clinical and control samples
3. For N reactions, add to a new tube:

   10·(N+1) µl of PCR-mix-1-FL-F with the corresponding name (see Table 2),

   5·(N+1) µl of PCR-mix-2-FRT and

   0.5·(N+1) µl of polymerase (TaqF) (scheme of reaction mixture preparation is specified in Table 3).

Table 3

Scheme of reaction mixture preparation

Reagent volume per 1 reaction (µl)	**Reagent volume for specified number of reactions (µl)**		
	10.0	**5.0**	**0.5**
The number of reactions*	**PCR-mix-1-FL-F**	**PCR-mix-2-FRT**	**Polymerase (TaqF)**
6	**60**	**30**	**3.0**
8	**80**	**40**	**4.0**
10	**100**	**50**	**5.0**
12	**120**	**60**	**6.0**
14	**140**	**70**	**7.0**
16	**160**	**80**	**8.0**
18	**180**	**90**	**9.0**
20	**200**	**100**	**10.0**
22	**220**	**110**	**11.0**
24	**240**	**120**	**12.0**
26	**260**	**130**	**13.0**
28	**280**	**140**	**14.0**
30	**300**	**150**	**15.0**
32	**320**	**160**	**16.0**

* Number of test samples including the control of extraction stage (N), controls of amplification, and one extra reaction (N+3+1).

4. Vortex the tube, then centrifuge it briefly.
5. Transfer **15 µl** of the prepared mixture to each tube.
6. Add **10 µl** of **cDNA** obtained at the RNA reverse transcription stage into the prepared tubes.
7. Carry out the control reactions (for each PCR-mix-1-FL-F, see Table 2):

NCA	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
C+	Add **10 µl of Positive Control** to tubes labeled C+ (**C+**$_{hRSv\text{-}hMpv}$ or other, depending on the PCR-mix-1-FL-F).
CS+	Add **10 µl of Positive Control STI-88** to the tube labeled CS+.
C–	Add **10 µl** of the sample extracted from **Negative Control** to the tube labeled C– (Negative control of Extraction).

8. Precipitate the reaction mixture in the bottom of the tube by short centrifuging (1-2 s).

8.3.2 Amplification

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

Table 4

ARVI-screen amplification program

	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)			**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	10 s	10	95	10 s	10
	54	20 s		54	25 s	
	72	10 s		72	25 s	
3	95	10 s	35	95	10 s	35
	54	20 s Fluorescence acquiring		54	25 s Fluorescence acquiring	
	72	10 s		72	25 s	

Fluorescent signal is detected in the channels for the FAM, JOE and ROX fluorophores.

NOTE: **It is not allowed** to perform «*Rhinovirus*» test together with other tests from eSens ARVI screen QL PCR kit when working with iCycler iQ and iQ5 instruments.

2. Insert tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

8.4 Instrument Settings

Test settings for rotor-type instruments

PCR-mix-1-FL / PCR-mix-1-FL-F	Channel	Calibrate/Gain Optimisation	Threshold	More Settings/ Outlier Removal	Slope Correct
hRSv - hMpv	*FAM/Green*	*from 5Fl to 10Fl*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is not used*
	ROX/Orange	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is used*
hAdv - hBov	*FAM/Green*	*from 5Fl to 10Fl*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is not used*
	ROX/Orange	*from 5Fl to 10Fl*	*0.05*	*3 %*	*Is used*
hRv	*FAM/Green*	*It is impossible to perform calibration using this mix*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow		-	-	-
	ROX/Orange		*0.1*	*5 %*	*Is used*
hPiv 1/3	*FAM/Green*	*from 5Fl to 10Fl*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow	*from 5Fl to 10Fl*	*0.1*	*10 %*	*Is used*
	ROX/Orange	*from 5Fl to 10Fl*	*0.1*	*10 %*	*Is not used*
hPiv 2/4	*FAM/Green*	*from 5Fl to 10Fl*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is not used*
	ROX/Orange	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is used*
hCov	*FAM/Green*	*from 5Fl to 10Fl*	*0.1*	*0 %*	*Is not used*
	JOE/Yellow	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is not used*
	ROX/Orange	*from 5Fl to 10Fl*	*0.1*	*5 %*	*Is not used*

Test settings for plate-type instruments

PCR-mix-1-FL / PCR-mix-1-FL-F	Channel	Threshold
All variants	FAM	Set the threshold line at the level corresponding to 10–20 % of maximum fluorescence obtained for the positive control, C+, during the last amplification cycle. Fluorescent curve should cross threshold line on the interval of exponential growth coming into linear growth
	HEX	
	ROX	

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instruments 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 required cDNA/DNA sample in the corresponding column of the result grid.

NOTE: Data analysis for each PCR-mix-1 should be performed individually, by selecting the area of the tubes which corresponds to the used PCR-mix-1. For the «*Rhinovirus*» (*hRv*) test analysis only the channels for **FAM** and **ROX** fluorophores should be used.

Table 5

Correspondence of PCR-mixes-1-FL-F and channels for ARVI pathogen detection

PCR-mix-1-FL-F	**Fluorescence detection**		
	FAM	**JOE**	**ROX**
hRSv-hMpv	IC	*hRSv*	*hMpv*
hAdv-hBov	IC	*hBov*	*hAdv*
hRv	IC	–	*hRv*
hPiv 1/3	IC	*hPiv* 3	*hPiv* 1
hPiv 2/4	IC	*hPiv* 2	*hPiv* 4
hCov	IC	*NL*-63, 229*E*	*HKU*-1, *OC* 43

Principle of interpretation is the following:

- DNA/RNA of an ARVI pathogen is **detected** if the *Ct* value for this sample is determined in the results grid in the corresponding channel. Moreover, the fluorescence curve for this sample should cross the threshold line in the area of exponential growth of the fluorescence.
- DNA/RNA of an ARVI pathogen is **not detected** if the *Ct* value for test sample is not determined (absent) in the results grid in the corresponding channel and if the *Ct* value in the results grid in the channel for FAM fluorophore does not exceed the specified boundary value.
- Result is **invalid** if the *Ct* for the test sample is not determined (absent) in the corresponding channel for ARVI pathogens (see Table 5) and if the *Ct* value in the channel for FAM fluorophore is absent or exceeds the specified boundary value. In such cases, the PCR analysis of the sample should be repeated starting from the DNA/RNA extraction stage.

NOTE: Boundary Ct values are specified in the Important Product Information Bulletin enclosed to the PCR kit. See also Guidelines [2]

The results of analysis are considered reliable only if the results obtained for Positive and Negative controls of amplification as well as for Negative control of extraction are correct (see Table 6 and 7).

Table 6

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**		
		FAM	**JOE**	**ROX**
		Detection of IC	Detection of ARVI pathogen	Detection of ARVI pathogen
C–	DNA/RNA extraction	< boundary value	Absent	Absent
NCA	PCR	Absent	Absent	Absent
CS+	PCR	< boundary value	Absent	Absent
C+	PCR	Absent	< boundary value*	< boundary value

* Positive Control cDNA *hRv* is not determined in the channel for JOE fluorophore.

Table 7

Boundary *Ct* values

Control samples	**Instrument**					
	Rotor-Gene 3000, 6000 etc.			**iCycler iQ, iCycler iQ5, CFX96**		
	***Ct* value**					
	FAM/Green	JOE/Yellow	ROX/Orange	FAM	JOE/HEX	ROX
NCA	-	-	-	-	-	-
C-	< 30	-	-	< 31	-	-
CS+	< 29	-	-	< 25	-	-
C+$_{hRSv\text{-}hMpv}$	-	< 24	< 24	-	< 25	< 25
C+$_{hAdv\text{-}hBov}$	-	< 22	< 24	-	< 24	< 24
C+$_{hRv}$	-	-	< 21	-	-	< 24
C+$_{hPiv\ 1/3}$	-	< 24	< 24	-	< 26	< 26
C+$_{hPiv\ 2/4}$	-	< 24	< 24	-	< 26	< 26
C+$_{hCov}$	-	< 22	< 22	-	< 22	< 22

Results for clinical samples

PCR-mix-1-FL / PCR-mix-1-FL-F	**Instrument**					
	Rotor-Gene 3000, 6000 etc.			**iCycler iQ, iCycler iQ5, CFX96**		
	***Ct* value**					
	FAM/Green	JOE/Yellow	ROX/Orange	FAM	JOE/HEX	ROX
	Detection of IC	Detection of pathogen	Detection of pathogen	Detection of IC	Detection of pathogen	Detection of pathogen
hRSv - hMpv	< 30	< 28	< 31	< 31	< 31	< 31
hAdv - hBov	< 30	< 28	< 31	< 31	< 29	< 30
hRv	< 30	–	< 27	< 31	–	< 30
***hPiv* 1/3**	< 30	< 31	< 30	< 31	< 32	< 30
***hPiv* 2/4**	< 30	< 30	< 30	< 31	< 30	< 30
hCov	< 30	< 30	< 30	< 31	< 32	< 30

10 TROUBLESHOOTING

1. If the *Ct* value for C+ is absent in the channels for JOE and/or ROX fluorophores or the *Ct* value is greater than the specified boundary value, PCR should be repeated for all negative clinical samples. If the same result is obtained, PCR analysis should be repeated for such samples starting from the DNA/RNA extraction stage.
2. If the *Ct* value for C– and/or NCA is present in the channel for ARVI pathogen detection, this means that reagents or samples are contaminated. Analysis should be repeated for all samples in which the ARVI pathogen DNA/RNA was detected starting from the DNA/RNA extraction stage and measures to detect and eliminate the source of contamination must be taken.

11 TRANSPORTATION

eSens ARVI screen 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 ARVI screen QL PCR kit** are to be stored at 2–8 °C (except for PCR-mix-2-FRT, PCR-mixes-1-FL-F (0.2 ml), and polymerase TaqF). All components of the **eSens ARVI screen QL PCR kit** are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FL-F hRSv – hMpv, PCR-mix-1-FL-F hPiv 1/3, PCR-mix-1-FL-F hPiv 2/4, PCR-mix-1-FL-F hCov, PCR-mix-1-FL-F hAdv – hBov and PCR-mix-1-FL-F hRv are to be kept away from light.

NOTE: PCR-mix-2-FRT, PCR-mixes-1-FL-F (0.2 ml), and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C

13 SPECIFICATIONS

13.1 Sensitivity

For samples from nasal and oropharyngeal swabs:

Pathogen	RNA/DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml*
hRSv	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^3$
hMpv	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^3$
hPiv	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^3$
hCov	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^4$
hBov	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^3$
hAdv	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$5x10^3$
hRv	RIBO-prep, ePure Viral nucleic acid kit	ES3304A	$1x10^3$

* Analytical sensitivity is expressed in genome equivalents (GE) of pathogen per 1 ml of sample.

13.2 Specificity

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

eSens ARVI screen QL PCR kit makes it possible to detect cDNA/DNA specific regions of ARVI causative agents listed above. The specificity of this kit was confirmed by investigation of the following reference strains: *human Respiratory Syncytial virus (*subgroup A, Long strain*), human Rhinoviruses (*13, 15, 16, 17, 21, 26, and 29 types). The specificity of the kit was also proved during examination of clinical material with subsequent confirmation by sequencing the amplification products of the following pathogens: *human Respiratory Syncytial virus* (types A and B); *Parainfluenza virus*-1-4*; human Coronaviruses* OC43, E229, NL63, and HKUI; *human Adenoviruses* B, C, and E; *Metapneumoviruses A* and *B;* and *human Bocavirus*. It is also possible to detect closely related variants of *enteroviruses* in the reaction for *rhinovirus* RNA detection. The adenovirus detection reaction is not intended for typing because of possible interaction with closely related adenoviruses of other types.

Non-specific reactions between the components of the PCR kit and cDNA/DNA of other viral (*Influenza A* and *B viruses,* Urbani SARS-associated *Coronavirus (*Frankfurt*), Coronaviruses* causing feline infectious peritonitis (F1, F2, and F5) and *s*wine transmissible gastroenteritis (TGEV1, TGEV8, and TGEV9), *Herpes viruses*, *Cytomegalovirus*, *Enteroviruses* (Echo9 and Echo30), and 60 samples of cerebrospinal fluid from meningitis patients containing *Enterovirus* RNA) and bacterial (*Streptococcus* spp., *Staphylococcus aureus*, *Mycoplasma influenza*, *Chlamydophila pneumonia*, *Haemophilus influenza, Moraxella catarrhalis,* and *Legionella*

pneumophila) agents that cause acute respiratory diseases as well as normal nasal and oropharyngeal human microflora and human cDNA/DNA are absent.

The clinical specificity of **eSens ARVI screen 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
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limit
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
	Use-by Date
	Consult instructions for use
	Keep away from sunlight
NCA	Negative control of amplification
C-	Negative control of extraction
C+	Positive control of amplification
IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

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

Mobil: +420 739 802 523