AmpliSens® Genoscreen-*IL*28B-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of the single-nucleotide polymorphism (SNP) rs8099917 and rs12979860 in the Interleukin-28B gene (*IL*28B) in the clinical material (whole blood or swabs collected from the internal cheek surface (buccal epithelium)) 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

The method is based on the extraction of total human DNA from the clinical material samples and amplification with real-time hybridization-fluorescence detection of human DNA fragments, containing SNP rs8099917 and rs12979860 in *IL*28B gene, and a fragment of human β-globin gene, which is used as an endogenous internal control (IC). Internal control is used in order to control all analysis stages and to estimate the influence of PCR inhibitors on analysis results.

The analysis of one sample is carried out in two tubes. SNP rs8099917 and internal control is detected in one of which tubes, SNP rs12979860 and internal control is detected in another one tube. The PCR kit allows differentiate homo- and heterozygous state for each test SNP. The PCR kit is developed for instruments with three or more channels for fluorescence detection.

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase by using 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	ROX
PCR-mix-1-FRT *IL*28B rs8099917			
DNA-target	DNA of rs8099917 locus	DNA of rs8099917 locus	Internal Control (IC) DNA
Target gene	The region including the **rs8099917** locus adjacent to the IL28B gene (human chromosome19)	The region including the **rs8099917** locus adjacent to the IL28B gene (human chromosome19)	Fragment of human β-globin gene
PCR-mix-1-FRT *IL*28B rs12979860			
DNA-target	DNA of rs8099917 locus	DNA of rs8099917 locus	Internal Control (IC) DNA
Target gene	The region including the **rs12979860** locus adjacent to the IL28B gene (human chromosome19)	The region including the **rs12979860** locus adjacent to the IL28B gene (human chromosome19)	Fragment of human β-globin gene

3. CONTENT

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit is produced in 1 form:
variant FRT-100 F REF R-O5-100-F(RG,iQ,Dt,CFX)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *IL*28B rs8099917	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-1-FRT *IL*28B rs12979860	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.6	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	2 tubes
Positive Control DNA *IL*28B rs8099917 ($C+_{rs17}$)	colorless clear liquid	0.2	1 tube
Positive Control DNA *IL*28B rs12979860 ($C+_{rs60}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.07	3 tubes
Negative control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see RIBO-prep, REF K2-9-Et-100-CE protocol).

Variant FRT-100 F is intended for 110 tests (220 amplification reactions), including controls.

4. ADDITIONAL REQUIREMENTS

- Transport medium for storage and transportation of respiratory swabs.
- Hemolytic reagent for selective lysis of blood erythrocytes during clinical material (whole peripheral and cord blood) pretreatment.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), CFX96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 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 with the range from 2 to 8 °C.
- Deep-freezer with the range 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

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

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (whole blood or swabs collected from the internal cheek surface (buccal epithelium)).

– *Whole blood*.
Collect 2.0 ml of blood to a tube with 0.2 ml of 3 % EDTA solution. Invert a closed tube 3-4 times to ensure proper mixing with EDTA. Blood samples can be stored at 2–8 °C for up to 48 h.

– *Swabs from the internal cheek surface (buccal epithelium).*
Swabs from the internal cheek surface (buccal epithelium) are taken with a sterile probe with a cotton tip. After swabbing, the probe should be placed to a 1.5-2.0-ml tube with cap with 0.5 ml of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE; REF 957-CE). The probe should be broken off at the score mark so that the tube is tightly closed. The sample can be stored at 2–8 °C for up to 3 days.

7. WORKING CONDITIONS

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- **RIBO-prep**, REF K1-2-50-CE.
  – Whole blood samples should be treated with **Hemolytic** (REF 137-CE) before adding the lysis solution. To do this, add 1.0 ml of "Hemolytic" and 100 µl of whole blood to a 1.5-ml tube. Close the tubes and carefully vortex. Incubate the tubes at room temperature for 5 min, vortex, and incubate for 5 min once again. Centrifuge (8,000 rpm, 2 min). Remove and discard the supernatant using a vacuum aspirator and a new tip for each sample. Leukocyte sediment should be lysed by addition of 300 µl of **Solution for Lysis**; otherwise, it should be stored frozen at the temperature from minus 24 to minus 16°C for 2 weeks and at or below minus 68°C for one year.
  – Prior to DNA extraction from swabs collected from the internal cheek surface and placed in **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE; REF 957-CE), thoroughly mix, and then centrifuge the samples briefly. Add 100 µl of clinical samples to 1.5-ml tubes and then add 300 µl of **Solution for Lysis**.

NOTE:

Extract the DNA according to the manufacturer's protocol taking into account the samples treatment described above and the correctives:

– After drying the pellet add 100 µl (instead of 50 µl) of RNA-buffer to the pellet.

The purified DNA can be stored at 2–8 °C for one week, at the temperature from minus 24 to minus 16 °C for 1 year, at the temperature not more than minus 68 °C for a long time.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

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

NOTE: Mix the components of the reaction mixture just before PCR-analysis.

1. Thaw the required number of tubes. Vortex the tubes and then centrifuge briefly to sediment drops.
2. Take the required number of tubes or strips for amplification of the DNA obtained from clinical and control samples (one control of DNA extraction, two controls of amplification).

NOTE: Each sample is analyzed with the use of two reaction mixtures, so it is necessary to take two tubes for each sample.

3. Take two 0.5 ml tubes for reaction mixtures preparation. Mark the tubes as **"rs17"** and **"rs60"**.
4. For 1 reactions, mix in the tube marked as **"rs17"**:
   5 µl of PCR-mix-2-FRT,
   0.5 µl of polymerase (TaqF),
   10 µl of PCR-mix-1-FRT *IL*28B rs8099917 (see also table 1).
5. For 1 reactions, mix in the tube marked as **"rs60"**:
   5 µl of PCR-mix-2-FRT,
   0.5 µl of polymerase (TaqF),
   10 µl of PCR-mix-1-FRT *IL*28B rs12979860 (see also table 2).

NOTE: Mix the reaction mixture with a reserve for one extra reaction (see table 2).

6. Mix the prepared mixtures and sediment the drops by short centrifugation using vortex.
7. Transfer **15 µl** of the prepared mixture to each tube for amplification so that the reaction mixture **"rs17"** is to be added to the tubes for amplification of fragment containing SNP rs8099917, the reaction mixture **"rs60"** is to be added to the tubes for amplification of fragment containing SNP rs12979860.

8. Add **10 µl** of **DNA samples** extracted from test and control samples, each sample including negative control of extraction (C–) is to be added to two tubes containing reaction mixtures **"rs17"** and **"rs60"**.
9. Carry out the control amplification reactions:
   NCA – Add **10 µl** of **TE-buffer** to the tubes with reaction mixtures **"rs17"** and **"rs60"** labeled NCA (Negative Control of Amplification).
   C+$_{rs17}$ – Add **10 µl** of **Positive Control DNA *IL*28B rs8099917 (C+$_{rs17}$)** to the tube labeled C+$_{rs17}$.
   C+$_{rs60}$ – Add **10 µl** of **Positive Control DNA *IL*28B rs12979860 (C+$_{rs60}$)** to the tube labeled C+$_{rs60}$.

Table 2

Scheme of reaction mixture preparation for variant FRT-100 F

Reagent volume per one reaction, µl		10.00	5.00	0.50
Number of clinical samples	Number of reactions[1]	PCR-mix-1-FRT[2]	PCR-mix-2-FRT[2]	Polymerase (TaqF)[2]
4	8	90	45	4.5
5	9	100	50	5.0
6	10	110	55	5.5
7	11	120	60	6.0
8	12	130	65	6.5
9	13	140	70	7.0
10	14	150	75	7.5
11	15	160	80	8.0
12	16	170	85	8.5

8.2.2. Amplification

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

Table 3

Amplification program

	Rotor-type instruments[3]			Plate-type instruments[4]		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
3	**95**	5 s	40	**95**	5 s	40
	60	40 s Fluorescence acquiring		**60**	50 s Fluorescence acquiring	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.

NOTE: First well of reaction module of rotor-type instruments is to be filled by the tube of current run containing reaction mixture "rs17".

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels. The channels for detection of nucleotide variants of analyzed SNP and internal control are specified in table 4.

Table 4

Reaction mixture	"rs17"	"rs60"
Channel for the fluorophore	Nucleotides being detected	
FAM	T	T
JOE	G	C
ROX	Internal Control (IC)	Internal Control (IC)

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 cDNA sample in the corresponding column of the results grid.
The amplification result in the channel is considered **positive** if the fluorescence curve crosses one time with threshold line in the area of reliable fluorescence growth, **negative** if the curve does not cross the threshold line (there is no *Ct* or *Cp* values) and **equivocal** in any other cases.

NOTE: Results interpretation is carried out separately for each reaction mixture.

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

Results interpretation of control samples
The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

Table 5

Reaction mixture	"rs17"			"rs60"		
Control	*Ct* value in the channel for fluorophore					
	FAM	JOE	ROX	FAM	JOE	ROX
C–	Absent	Absent	Absent	Absent	Absent	Absent
NCA	Absent	Absent	Absent	Absent	Absent	Absent
C+$_{rs17}$	<boundary value	<boundary value	<boundary value	—	—	—
C+$_{rs60}$	—	—	—	<boundary value	<boundary value	<boundary value

Note – "–" is not tested for this reaction mixture.
Results interpretation of test clinical samples (see also Table 6)

NOTE: Browse data for each PCR-mix-1 separately in each channel when using plate-type instruments.

[1] Number of clinical samples + 1 control of extraction stage + 3 controls of amplification (N+4, N – number of clinical samples).
[2] Reagent volumes are specified with a reserve for one extra reaction.
[3] For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene (QIAGEN, Germany).
[4] For example, iCycler iQ5 (Bio-Rad, USA), CFX96 (Bio-Rad, USA).

Table 5

PCR-mix-1	Channel for the fluorophore			Detected genotype
	FAM (T)	**JOE (G)**	**ROX (IC)**	
PCR-mix-1-FRT " **rs17**"	+	-	*Ct* value < boundary value	**TT**
	-	+	*Ct* value < boundary value	**GG**
	for **rotor-type** instruments			
	+ *Ct* (FAM) **>** *Ct* (JOE)	+ *Ct* (FAM) **>** *Ct* (JOE)	*Ct* value < boundary value	**TG**
	+ *Ct* (FAM) **<** *Ct* (JOE)	+ *Ct* (FAM) **<** *Ct* (JOE)	*Ct* value < boundary value	**TT**
	for **plate-type** instruments			
	+ *Ct* (FAM) – *Ct* (ROX) **< N**	+ *Ct* (JOE) – *Ct* (ROX) **< N**	*Ct* value < boundary value	**TG**
	+ *Ct* (FAM) - *Ct* (ROX) **> N**	+	*Ct* value < boundary value	**GG**
	+	+ *Ct* (JOE) - *Ct* (ROX) **> N**	*Ct* value < boundary value	**TT**
	FAM (T)	**JOE (C)**	**ROX (IC)**	**Detected genotype**
PCR-mix-1-FRT " **rs60**"	+	-	*Ct* value < boundary value	**TT**
	-	+	*Ct* value < boundary value	**CC**
	for **rotor-type** instruments			
	+ *Ct* (FAM) **>** *Ct* (JOE)	+ *Ct* (FAM) **>** *Ct* (JOE)	*Ct* value < boundary value	**CT**
	+ *Ct* (FAM) **<** *Ct* (JOE)	+ *Ct* (FAM) **<** *Ct* (JOE)	*Ct* value < boundary value	**TT**
	for **plate-type** instruments			
	+ *Ct* (FAM) – *Ct* (ROX) **< N**	+ *Ct* (JOE) – *Ct* (ROX) **< N**	*Ct* value < boundary value	**CT**
	+ *Ct* (FAM) - *Ct* (ROX) **> N**	+	*Ct* value < boundary value	**CC**
	+	+ *Ct* (JOE) - *Ct* (ROX) **> N**	*Ct* value < boundary value	**TT**

NOTE: Boundary *Ct* values and N are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

A. Interpretation of results, obtained with the use of reaction mixture "rs17"

1. If in the results grid for the sample with given reaction mixture the *Ct* value is defined only in the channels for the **FAM and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then the result for SNP **rs8099917** is given as **"Genotype TT is detected".**
2. If in the results grid for the sample with given reaction mixture the *Ct* value is defined only in the channels for the **JOE and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then the result for SNP **rs8099917** is given as **"Genotype GG is detected".**
3. If in the results grid for the sample with given reaction mixture the *Ct* value is defined in the channels for the **FAM, JOE and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then:
   a) with the use of rotor-type instruments the result is given as **"Genotype TG is detected"** only if the *Ct* value in the channel for the **FAM** fluorophore exceeds *Ct* value in the channel for the **JOE** fluorophore. If the *Ct* value in the channel for the **FAM** fluorophore is less than the *Ct* value in the channel for the **JOE** fluorophore then the result in the channel for the **JOE** fluorophore is not interpreted and the result is given as **"Genotype TT is detected";**
   b) with the use of plate-type instruments the result is given as **"Genotype TG is detected"** only if the *Ct* value in each channel for the **FAM** or **JOE** fluorophores exceeds the *Ct* value in the channel for the **ROX** fluorophore **not more than N cycles**[5]. If in one of the channels for the **FAM** or **JOE** fluorophores the *Ct* value exceeds the *Ct* value in the channel for the **ROX** fluorophore **more than N cycles,** then the result in this channel is not interpreted and the result is given as **"Genotype GG is detected"** or **"Genotype TT is detected".**
4. If in the results grid for the sample with given reaction mixture the *Ct* value is not defined in the channels for the **FAM** and **JOE** fluorophores then it is necessary to repeat the PCR analysis for this sample beginning from the DNA extraction stage.
5. If in the results grid for the sample with given reaction mixture the *Ct* value in the channel for the **ROX** fluorophore exceeds the value specified in the *Important Product Information Bulletin* (regardless of the results obtained in the channels for the **FAM** and **JOE** fluorophores) then it is necessary to repeat the PCR analysis for this sample beginning from the DNA extraction stage.

B. Interpretation of results, obtained with the use of reaction mixture "rs60"

1. If in the results grid for the sample with given reaction mixture the *Ct* value is defined only in the channels for the **FAM and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then the result for SNP **rs12979860** is given as **"Genotype TT is detected".**
2. If in the results grid for the sample with given reaction mixture the *Ct* value is defined only in the channels for the **JOE and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then the result for SNP **rs12979860** is given as **"Genotype CC is detected".**
3. If in the results grid for the sample with given reaction mixture the *Ct* value is defined in the channels for the **FAM, JOE and ROX** fluorophores and the *Ct* value does not exceed the value specified in the *Important Product Information Bulletin* in the channel for the **ROX** fluorophore, then:
   a) with the use of rotor-type instruments the result is given as **"Genotype CT is detected"** only if the *Ct* value in the channel for the **FAM** fluorophore exceeds *Ct* value in the channel for the **JOE** fluorophore. If the *Ct* value in the channel for the **FAM** fluorophore is less than the *Ct* value in the channel for the **JOE** fluorophore then the result in the channel for the **JOE** fluorophore is not interpreted and the result is given as **"Genotype TT is detected";**
   b) with the use of plate-type instruments the result is given as **"Genotype CT is detected"** only if the *Ct* value in each channel for the **FAM** or **JOE** fluorophores exceeds the *Ct* value in the channel for the **ROX** fluorophore **not more than N cycles**[5]. If in one of the channels for the **FAM** or **JOE** fluorophores the *Ct* value exceeds the *Ct* value in the channel for the **ROX** fluorophore **more than N cycles,** then the result in this channel is not interpreted and the result is given as **"Genotype CC is detected" or "Genotype TT is detected".**
4. If in the results grid for the sample with given reaction mixture the *Ct* value is not defined in the channels for the **FAM** and **JOE** fluorophores then it is necessary to repeat the PCR analysis for this sample beginning from the DNA extraction stage.
5. If in the results grid for the sample with given reaction mixture the *Ct* value in the channel for the **ROX** fluorophore exceeds the value specified in the *Important Product Information Bulletin* (regardless of the results obtained in the channels for the **FAM** and **JOE** fluorophores) then it is necessary to repeat the PCR analysis for this sample beginning from the DNA extraction stage.

NOTE: Analysis and interpretation of results, obtained for test clinical samples depending on type of the instruments, is detailed in Guidelines [2].

10. TROUBLESHOOTING

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

1. If the *Ct* value is absent for both controls C+$_{rs17}$ and C+$_{rs60}$ then the PCR analysis should be repeated for all samples beginning from the amplification stage.
2. If the *Ct* value exceeds the value specified in the *Important Product Information Bulletin* in at least one sample with positive control of amplification (C+$_{rs17}$ or C+$_{rs60}$) the PCR analysis (beginning from the amplification stage) should be repeated for all samples with PCR-mix-1 for which incorrect results was obtained for corresponding C+.
3. If the positive signal is registered for negative control of extraction (C–) and/or negative control of amplification (NCA) with any reaction mixture in the channels for the **FAM** and **JOE** fluorophores the PCR analysis (beginning from the amplification stage) should be repeated for all samples in which the *Ct* value was detected in the results grid in given channel for this reaction mixture.

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

11. TRANSPORTATION

AmpliSens® Genoscreen-*IL*28B-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® Genoscreen-*IL*28B-FRT** PCR kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® Genoscreen-*IL*28B-FRT** PCR 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: PCR-mix-1-FRT *IL*28B rs8099917 and PCR-mix-1-FRT *IL*28B rs12979860 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Extraction volume, µl	Clinical material	Sensitivity, copies/ml
100	Whole blood, swabs collected from the internal cheek surface (buccal epithelium)	$5x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® Genoscreen-*IL*28B-FRT** 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.

Estimation of PCR kit analytical specificity showed the absence of cross reactions between detected alleles within each test SNP with the use of high concentrated positive control samples and clinical samples.

The clinical specificity of **AmpliSens® Genoscreen-*IL*28B-FRT** PCR kit was confirmed in laboratory clinical trials.

14. 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.
2. Guidelines to the **AmpliSens® Genoscreen-*IL*28B-FRT** PCR kit for qualitative detection of the single-nucleotide polymorphism (SNP) rs8099917 and rs12979860 in the Interleukin-28B gene (*IL*28B) in the clinical material (whole blood or swabs collected from the internal cheek surface (buccal epithelium)) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® Genoscreen-*IL*28B-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

[5] N value for each type of instruments is specified in Guidelines [2] and *Important Product Information Bulletin*.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.03.19 DV	3. Content	The color of the reagent was specified
10.06.20 VA	Through the text	The text formatting was changed
	Footer	The phrase “Not for use in the Russian Federation” was added
	2. Principle of PCR detection	The table with targets was added
17.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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