AmpliSens® Genoscreen HLA B*5701-FRT PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
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 HLA B*5701-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of B locus 5701 allele of human major histocompatibility complex (HLA B*5701) in the clinical material (whole blood and oropharyngeal swabs) 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

HLA B*5701 allele detection includes:

a) Total DNA extraction.

b) Real-time PCR of a region of major histocompatibility complex B locus and a fragment of human β-globin gene, which is used as an endogenous internal control. HLA B*5701 allele is detected by using allele-specific oligonucleotides; therefore, positive result of amplification (accumulation of fluorescent signal) indicates the presence of HLA B*5701 and does not require further analysis of the sample.

Amplification of a fragment of human β-globin gene, which is used as an endogenous internal control, allows monitoring of sample collection, handling, and storage.

Positive test result will be registered if HLA B*5701 allele is either homo- or heterozygous. This PCR kit does not allow discrimination between homozygous and heterozygous alleles. Take into account that a positive result may be due to rarely occurring closely related alleles, such as B*5514, B*5706, B*5708, B*5710, B*5713-B*5716, B*5718, B*5719, and B*5814 (less than 0.1 %).

AmpliSens® Genoscreen HLA B*5701-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). 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
DNA-target	β-globin gene	alleles 5701 of B gene
Target gene	β-globin gene	alleles 5701 of B gene

3. CONTENT

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is produced in 1 form:

variant FRT REF R-O2(RG,iQ)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT HLA	clear liquid from colorless to light lilac colour	0.6	2 tubes
RT-PCR-mix-2-FL	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TE-buffer	colorless clear liquid	0.07	2 tubes
Positive Control DNA HLA B*5701 and human DNA ($C+_{HLA\ B*5701}$)	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	0.5	4 tubes

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

Variant FRT is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (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 3000/6000 (Corbett Research, Australia); iCycler iQ or iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.2- or 0.1-ml):
  a) 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps (nonstriped) 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 barriers 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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 of biological material samples for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from:

—*Whole blood*

Collect 2 ml of blood into the tube with 0.2 ml of 3% EDTA solution. Invert the closed tube several times to ensure proper mixing. Blood samples should be stored at 2–8 °C for up to 48 h.

—*Oropharyngeal swabs*

Oropharyngeal swabs are taken with a sterile probe with a cotton tip. After swabbing, the probe should be placed into the tube with 0.5 ml of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 958-CE, REF 959-CE). The probe should be broken off at the score mark so that the tube is tightly closed. The sample should be stored at 2–8 °C for up to 3 days.

7. WORKING CONDITIONS

AmpliSens® Genoscreen HLA B*5701-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 K2-9-Et-100-CE.

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

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

NOTE: Extract the DNA according to the manufacturer's protocol.

NOTE: 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 0.1 ml of whole blood into a 1.5-ml tube. 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. Leukocyte sediment should be immediately lysed; otherwise, it should be stored frozen at or below minus 16°C for up to 2 weeks or at or below minus 68°C for a long time.

NOTE: Prior to DNA extraction from oropharyngeal swabs placed in **Transport Medium for Storage and Transportation of Respiratory Swabs** thoroughly mix, and then briefly vortex the samples.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1. Take the required number of tubes for amplification for the clinical and control samples. The type of tubes, strips and plates depends on the PCR instrument used for analysis.
2. To prepare the reaction mixture, mix **10 µl of PCR-mix-1-FRT HLA, 5 µl of RT-PCR-mix-2-FL, 0.5 µl of polymerase (TaqF)** per one reaction in a new sterile tube.

Add one extra reaction when calculating the reaction mixture volume (see table 2).

Table 2

Number of samples	Volume of the reagents for specified number of samples, µl (one extra reaction is included)		
	PCR-mix-1-FRT HLA	RT-PCR-mix-2-FL	Polymerase (TaqF)
6	70	35	3.5
11	120	60	6.0
18	190	95	9.5

3. Thoroughly vortex prepared mixture, make sure there are no drops on the wall of the tubes.
4. Transfer **15 µl** of prepared reaction mix to each tube. Discard the rest of the mixture.
5. Using tips with aerosol filter, add **10 µl** of **DNA samples** obtained at the stage of DNA extraction into prepared tubes.
6. Carry out control amplification reactions:

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

C+ - Add **10 µl** of **Positive Control DNA HLA B*5701 and human DNA ($C+_{HLA\ B*5701}$)** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl** of the **sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

8.2.2. Amplification

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

Table 3

Amplification program for rotor-type instruments[1]

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
3	95	5 s	–	40
	60	40 s	FAM, JOE	

Table 4

Amplification program for plate-type instruments [2]

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
3	95	5 s	–	40
	60	50 s	FAM, JOE	

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

[1] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia)

[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA)

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:

– The signal of the IC DNA (human β-globin gene) amplification product is detected in the channel for the FAM fluorophore.
– The signal of the HLA B*5701 DNA amplification product is detected in the channel for the JOE fluorophore.

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, and the *Ct* value for this channel is less than the value specified in the *Important Product Information Bulletin*. The amplification result in the channel is considered **negative** in case of absence of the curve with typical shape and it does not cross the threshold line (there is no *Ct* or *Cp* values). The result is equivocal in any other cases.

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

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	DNA extraction	> boundary value[3] or absent	absent
NCA	PCR	absent	absent
C+	PCR	< boundary value	< boundary value

Results interpretation of test clinical samples

– The sample is considered to be **positive** if a positive result is obtained in the channel for the JOE fluorophore and the *Ct* value exceeds the value in the channel for the FAM fluorophore not more than 5 cycles.
– The sample is considered to be **negative** if either a negative result is obtained in the channel for the JOE fluorophore or the *Ct* value exceeds the value in the channel for the FAM fluorophore more than 5 cycles.

10. TROUBLESHOOTING

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

1. If the positive signal is absent in any channel for the Positive Control of amplification (C+), this may be due to errors in the PCR run. PCR should be repeated.
2. If the *Ct* value is absent for the clinical sample in the channel for IC detection, this may be due to incorrect clinical material pretreatment and subsequent loss of DNA or inhibition of PCR. The analysis should be repeated starting from the DNA extraction stage.
3. If *Ct* value determined for the clinical sample in the channel for IC detection is greater than the *Ct* value specified in the *Important Product Information Bulletin*, the sample is considered **equivocal**. This may be due to incorrect clinical material pretreatment and subsequent loss of DNA or inhibition of PCR. The analysis should be repeated starting from the DNA extraction stage.
4. If the positive signal is detected for the Negative Control of amplification (NCA) in any channel, this indicates the contamination of reagents or samples. In this case, the results of analysis of all samples are considered **invalid**. It is necessary to repeat the analysis and to take measures to detect and eliminate the source of contamination.
5. If the positive signal is detected for the Negative Control of extraction (C–) in the channel for the JOE fluorophore (HLA B*5701) or the Ct value determined in the channel for the FAM fluorophore (IC) is less than the value specified in the *Important Product Information Bulletin*, the results of analysis of all samples are considered **invalid**. It is necessary to repeat the analysis and to take measures to detect and eliminate the source of contamination.

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

11. TRANSPORTATION

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 ºC when not in use. All components of the **AmpliSens® Genoscreen HLA B*5701-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 HLA is to be kept away from light.

[3] If the *Ct* value determined for the Negative Control of extraction (C–) in the channel for the FAM fluorophore is greater than the *Ct* value specified in the *Important Product Information Bulletin*, measures to detect and eliminate the source of contamination should be taken.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit is 1 x 10^3 cells/ml.

NOTE: The claimed analytical features of **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit are guaranteed only when additional reagents kit RIBO-prep (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") is used.

13.2. Specificity

The analytical specificity of **AmpliSens® Genoscreen HLA B*5701-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. The clinical specificity of **AmpliSens ® Genoscreen HLA B*5701-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 HLA B*5701-FRT** PCR kit for qualitative detection of B locus 5701 allele of human major histocompatibility complex (HLA B*5701) in the clinical material 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 HLA B*5701-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
18.09.15 ME	Text	Corrections according to the template
	8. Protocol	Information about carrying out the Negative control of extraction was added
	9. Data analysis	The sections were rewritten. Information about result interpretation and troubleshooting for C– was corrected
	10. Troubleshooting	
	14. References	The references to guidelines was added
27.12.17 ME	3. Content	The color of the reagent was specified
05.12.18 EM	2. Principle of PCR detection	The table with targets was added
	Through the text	The text formatting was changed
27.02.20 PM	Footer	The phrase "Not for use in the Russian Federation" was added
01.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

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