CE
1023

IVD

For Professional Use Only

AmpliSens® Genoscreen HLA B*5701-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

TABLE OF CONTENTS

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 clinical materials (whole blood and oropharyngeal swabs) by means of real-time hybridization-fluorescence detection.

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.

3. CONTENT

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

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit variant FRT (for use with RG, iQ)

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

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Amount*
PCR-mix-1-FRT HLA	colorless clear liquid	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+$_{\text{HLA B*5701}}$)	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.

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves.
- 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.
- Personal thermocycler (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ or iQ5 (Bio-Rad, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.1-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.

- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining 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 to a tube with 0.2 ml of 3% EDTA solution. Invert a 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 to a tube with 0.5 ml of “Transport Medium for Storage and Transportation Respiratory Swabs” (**REF** 958-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 that the following nucleic acid extraction kit is used:

- RIBO-prep, **REF** K2-9-Et-100-CE.

Extract DNA according to the manufacturer’s instruction.

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 to 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 3 days or at or below minus 68°C for a long time.

Prior to DNA extraction from throat swabs placed in “Transport Medium for Storage and Transportation of Respiratory Swabs” (**REF** 957-CE), 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. Prepare the **reaction mixture**. Per **one** reaction:
   - **10 µl of PCR-mix-1-FRT HLA**
   - **5 µl of RT-PCR-mix-2-FL**
   - **0.5 µl of polymerase (TaqF)**

Add one extra reaction when calculating the reaction mixture volume.

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**

2. Thoroughly vortex prepared mixture, make sure there are no drops on the wall of the

tubes.

3. Take the required number of the PCR tubes for amplification of clinical and control samples. Transfer **15 µl** of prepared reaction mix to each tube.

4. **Add 10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.

5. 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** to the tube labeled C+ (Positive Control of Amplification).

8.2.2. Amplification

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

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/Green, JOE/Yellow,	

2. Adjust the fluorescence channel sensitivity according to Guidelines.

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	–	
4	95	5 s	–	40
	60	50 s	FAM, JOE/HEX	

2. Adjust the fluorescence channel sensitivity according to Guidelines.

9. DATA ANALYSIS

Internal Control (fragment of human β-globin gene) is detected in the FAM/Green fluorescence channel, HLA B*5701 DNA is detected in the JOE/HEX/Yellow fluorescence channel.

See **Guidelines** for data analysis settings for Rotor-Gene 3000/6000 and iQ5/iCycler iQ

[1] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia)
[2] For example, iCycler iQ, iQ5 (Bio-Rad, USA)

Instruments, respectively.

Interpretation of results

The results are interpreted by the software of the PCR instrument by the crossing (or not crossing) of fluorescence curve with the threshold line.

The results of analysis are considered reliable only if the results obtained for both Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct.

Results for controls

Control	Stage for control	Ct in channel		Interpretation
		FAM /Green	JOE/Yellow/HEX	
C–	DNA extraction	Neg	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	OK

*Boundary values are specified in the *Important Product Information Bulletin.*

1. The sample is considered to be **positive** if a positive result is obtained in the JOE/HEX/Yellow channel and the Ct value is at most **5** cycles greater than the Ct value in the FAM/Green channel.
2. The sample is considered to be **negative** if either a negative result is obtained in the JOE/HEX/Yellow channel or the Ct value detected is at least **5** cycles greater than the Ct value in the FAM/Green channel**.**

10. TROUBLESHOOTING

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

1. If no signal is detected for the Positive Control of amplification in any channels, this may due to errors in the PCR run. PCR should be repeated.
2. If no Ct value is detected for a clinical sample in the channel for the Internal Control detection, this may be due to incorrect processing of clinical material and ultimate loss of DNA or inhibition of PCR. The analysis should be repeated starting from the DNA extraction stage.
3. If Ct value of a clinical sample obtained in the channel for detection of the Internal Control is greater than the Ct value specified in the *Important Product Information Bulletin*, the sample is considered **equivocal**. This may be due to incorrect processing of clinical material and ultimate loss of DNA or inhibition of PCR. The analysis should be repeated starting from the DNA extraction stage.
4. If a signal is detected in the Negative Control of extraction (C–) or 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.

If you have any further questions or if 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 expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FRT HLA is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit is not less than 1 x 10^3 cells per 1 ml of a sample (cells/ml).

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

Specificity of **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. 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, Moscow, 2008.

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 **AmpliSens®** **Genoscreen HLA B*5701-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)	**IC**	Internal control
iQ	For working with iQ5, iCycler iQ (Bio-Rad)		

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”