IVD For *in Vitro* Diagnostic Use

CE

HSV I/II Typing Real-TM

Handbook

Real Time PCR kit for qualitative detection and differentiation of *Herpes Viruses I and II*

REF V38-100FRT

REF TV38-100FRT

Σ 100

NAME

HSV I/II Typing Real-TM

INTRODUCTION

Herpes simplex virus-1 and -2 belong to the *Alpha-herpesvirinae* of the family *Herpesviridae*. Their genomes consist of double-stranded DNAs. HSV is one of the most widespread viruses with a prevalence of 95 % and a presence of HSV-2 in 30 – 50 % of human population.

HSV-1, the so called oral strain, causes herpes labialis, herpetic stomatitis and keratitis. HSV-2 is called genital strain and causes predominantly genital herpes. In terms of localization of herpes lesions, two forms of herpes are distinguished, the labial herpes with herpes lesions on the upper part of the body (lips, face, mucous membranes of the mouth); and the genital herpes with herpes lesions on the lower part of the body (mucous membranes of the genitals, buttocks, legs). It was believed in the past that only HSV-I could induce labial herpes and only HSV-II could induce genital herpes. The present data disprove this point of view demonstrating tropism common for both types of the virus.

The method of polymerase chain reaction (PCR) in the HSV diagnosis is highly sensitive, specific and less laborious and it has become very important in the laboratory diagnostics of HSV.

INTENDED USE

kit **HSV I/II Typing Real-TM** is a test for the qualitative detection and differentiation of Herpes Simplex Virus type I and Herpes Simplex Virus type II DNA in the whole blood, plasma, liquor, urogenital swabs, urine, prostatic liquid and other biological samples.

PRINCIPLE OF ASSAY

kit **HSV I/II Typing Real-TM** is based on two major processes: isolation of DNA from specimens and Real Time amplification. Herpes Simplex Virus DNA is extracted from the specimens, amplified using Real-Time amplification and detected fluorescent reporter dye probes specific for *HSV* DNA I, II and Internal Control. Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. IC is detected in a channel other than the *HSV*.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (V38-100FRT)

Part N° 2 – **"HSV I/II Typing Real-TM":** Real Time amplification

- **PCR-mix-1-FRT**, 1,2 ml;
- **PCR-Buffer-FRT**, 2 x 0,35 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;
- **Pos C+**, 0,2 ml;
- **Negative Control C-**, 1,2ml;*
- **Internal Control IC**, 1,0 ml;**
- **DNA-buffer**, 0,5 ml;

Contains reagents for 110 tests.

Module No.2:Complete Real Time PCR test with DNA purification kit(TV38-100FRT)

Part N° 1 – **"DNA-sorb-B":** Sample preparation

- **Lysis Solution**, 2 x15 ml;
- **Washing Solution 1**, 2 x 15 ml;
- **Washing Solution 2**, 2 x 50 ml;
- **Sorbent**, 2 x 1,25 ml;
- **DNA-eluent**, 2 x 5 ml.

Contains reagents for 100 tests.

Part N° 2 – **"HSV I/II Typing Real-TM":** Real Time amplification

- **PCR-mix-1-FRT**, 1,2 ml;
- **PCR-Buffer-FRT**, 2 x 0,35 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;
- **Pos C+**, 0,2 ml;
- **Negative Control C-**, 1,2ml;*
- **Internal Control IC**, 1,0 ml;**
- **DNA-buffer**, 0,5 ml;

Contains reagents for 110 tests.

**must be used in the isolation procedure as Negative Control of Extraction.*
***add 10 µl of Internal Control during the DNA isolation directly to the sample/lysis mixture (see DNA-Sorb-B REF K-1-1/B protocol).*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- DNA extraction kit (Module No. 1)
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- Dry heat block
- Vortex mixer
- Pipettes
- Sterile pipette tips with filters
- 1,5 ml polypropylene sterile tubes
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: Real Time amplification:

- Real Time Thermal cycler
- Reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Freezer, refrigerator

STORAGE INSTRUCTIONS

HSV I/II Typing Real-TM must be stored at 2-8°C. **TaqF Polymerase** must be stored at -20°C. **DNA-sorb-B** must be stored at 2-8°C. The kits can be shipped at 2-8°C for 3-4 days but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

HSV I/II Typing Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

In accordance with Sacace’s ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- ⚠ Lysis Solution contains guanidine thiocyanate*. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- Use sterile pipette tips with aerosol barriers 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 authorities' 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 other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes 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.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move 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.

**** Only for Module No.2***

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (EN375). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HSV I/II Typing Real-TM can analyze DNA extracted with **DNA-Sorb-B** (REF K-1-1/B) from:

- *whole blood* collected in either ACD or EDTA tubes;
- *plasma* collected in either ACD or EDTA tubes;
- *liquor* stored in “Eppendorf” tube;
- *tissue:* 1,0 gr homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile. Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- *prostatic liquid* stored in “Eppendorf” tube;
- *seminal liquid: t*ransfer about 30 µl of seminal liquid to a polypropylene tube (1,5 ml) and add 70 µl of sterile saline solution;
- *cervical, urethral, conjunctival swabs*:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Specimens can be stored at +2-8°C for no longer than 48 hours, or frozen at -20°C to -80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

* recommended kit for DNA extraction: DNA-Sorb-A (REF K-1-1/A)

DNA ISOLATION

The following kits are recommended:

- ⇨ **DNA-Sorb-B** (Sacace, REF K-1-1/B) for whole blood, liquor, tissue, etc;
- ⇨ **DNA-Sorb-A** (Sacace, REF K-1-1/A) for swabs;

Please carry out DNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control during DNA isolation procedure directly to the sample/lysis mixture.

(Note: the Sacace Internal Control is the same for all urogenital infection Real Time kits)

SPECIMEN AND REAGENT PREPARATION (reagents supplied with the Module No.2)

1. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60–65°C until disappearance of ice crystals. Prepare required quantity of 1.5 ml polypropylene tubes including one tube for **Negative Control of Extraction**.
2. Add to each tube **300 µl** of **Lysis Solution** and **10 µl** of **Internal Control.**
3. Add **100 µl** of **Samples** to the appropriate tube.
4. Prepare Controls as follows:
   - add **100 µl** of **C–** (**Neg Control** provided with the amplification kit) to the tube labeled C*neg*.
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 7-10 sec. If the sample is not completely dissolved it is recommended to re-centrifuge the tube for 5 min at a maximum speed (12000-16000 g.) and transfer the supernatant into a new tube for DNA extraction.
6. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
7. Vortex for 5-7 sec and incubate all tubes for 3 min at room temperature. Repeat this step.
8. Centrifuge all tubes for 30 sec at 8000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet.
9. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube.
10. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 30 sec at 8000g. Remove and discard supernatant from each tube.
11. Repeat step 10 and incubate all tubes with open cap for 5 min at 65°C.
12. Resuspend the pellet in **50 µl of DNA-eluent.** Incubate for 5 min at 65°C and vortex periodically.
13. Centrifuge the tubes for 1 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. If amplification is not performed in the same day of extraction, the processed samples can be stored at 2-8°C for at maximum period of 5 days or frozen at –20°/-80°C.

PROTOCOL:

1. Prepare required quantity of reaction tubes for samples (N) and controls (N+2).
2. Prepare in the new sterile tube for each sample **10*(N+1) µl** of **PCR-mix-1-FRT**, **5,0*(N+1)** of **PCR-Buffer-FRT** and **0,5*(N+1) µl** of **TaqF DNA Polymerase.** Vortex and centrifuge for 2-3 sec.
3. Add to each tube **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA** sample to appropriate tube. Mix by pipetting.
4. Prepare for each panel 2 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
   - add **10 µl** of **Positive Control C+** to the tube labeled Amplification Positive Control;
5. Insert the tubes in the thermalcycler.

Amplification

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

	Rotor-type Instruments[1]			**Plate- or modular type Instruments**[2]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] *For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, SaCycler-96™ (Sacace), CFX/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid), LineGeneK® (Bioer)*

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green, JOE/Yellow/Hex/Cy3 and Rox(Orange)/TexasRed fluorescence channels.

HSV II is detected on the FAM (Green) channel, *HSV I* is detected on the JOE(Yellow)/HEX/Cy3 channel, *IC DNA* on the *Rox(Orange)/TexasRed channel*

INSTRUMENT SETTINGS

Rotor-type instruments

Channel	Calibrate/Gain Optimisation…	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*from 5 Fl to 10 Fl*	*0.1*	*0 %*	*Off*
JOE/Yellow	*from 4 Fl to 8 Fl*	*0.1*	*5 %*	*Off*
Rox/Orange	*from 4 Fl to 8 Fl*	*0.1*	*5 %*	*Off*

Plate-type instruments

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

Boundary value of the cycle threshold, Ct

Sample	Channel	Ct for rotor-type instrument	Ct for plate-type instrument
C+	FAM	*33*	*36*
	JOE	*30*	*33*
	ROX	*33*	*36*
Samples, C–	ROX	*33*	*36*

DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– The signal from the *HSV II* DNA amplification product is detected in the FAM/Green channel;

– The signal from the *HSV I* DNA amplification product is detected in the JOE/Yellow/HEX/Cy3 channel;

– The signal from the Internal Control amplification product is detected in the Rox/Orange/TexasRed channel.

Interpretation of results

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

Principle of interpretation:

— *HSV II* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.

— *HSV I* DNA is **detected** in a sample if its Ct value is present in the Joe channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth

— *HSV I/II* DNA is **not detected** in a sample if its Ct value is absent in the FAM/Joe channels (fluorescence curve does not cross the threshold line) while the Ct value in the Rox channel is less than 33.

— The result is **invalid** if the Ct value of a sample in the FAM/Joe channels is absent while the Ct value in the Rox channel is either absent or greater than the specified boundary value (Ct > 36). It is necessary to repeat the PCR analysis of such samples.

The result of 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.

Control	Stage for control	Ct channel Fam	Ct channel Joe	Ct channel Rox	Interpretation
NCE	DNA isolation	NEG	NEG	POS	Valid result
NCA	Amplification	NEG	NEG	NEG	Valid result
C+	Amplification	POS	POS	POS	Valid result

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

SPECIFICATIONS

Sensitivity

The analytical sensitivity of **HSV I/II Real-TM Typing** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	DNA-sorb-A	5×10^2

** Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.*

Specificity

The analytical specificity of **HSV I/II Typing Real-TM** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. There were no nonspecific responses during examination of human DNA as well as DNA panel of the following microorganisms: *Mycoplasma hominis, Lactobacillus spp., Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Neisseria gonorrhoeae, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Chlamydia trachomatis, Trichomonas vaginalis, Gardnerella vaginalis, Toxoplasma gondii*, *CMV,* and *HPV.*

TROUBLESHOOTING

1. Weak or no signal of the IC (Rox/Orange/TexasRed channel) for the Negative Control of extraction.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
        - ⇒ Check the storage conditions
    - Improper DNA extraction.
        - ⇒ Repeat analysis starting from the DNA extraction stage
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
        - ⇒ Make attention during the DNA extraction procedure.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. Fam (Green) or Joe (Yellow) signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        - ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive control at last.
        - ⇒ Repeat the PCR preparation with the new set of reagents.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***