Sacace BIOTECHNOLOGIES

HIV DNA Real-TM

Handbook

Real-Time test for the Qualitative detection of the proviral DNA of human immunodeficiency virus (HIV)

REF R-V1-D

REF TR-V1-D

Σ 100

NAME

HIV DNA Real-TM

INTENDED USE

kit **HIV DNA Real-TM** is a Real-Time test for the Qualitative detection of the proviral DNA of human immunodeficiency virus (HIV) in whole blood.

PRINCIPLE OF ASSAY

Kit **HIV DNA Real-TM** is a Real-Time test for the Qualitative detection of Human Immunodeficiency Virus in whole blood. HIV DNA is extracted from blood, amplified using Real Time Amplification and detected using fluorescent reporter dye probes specific for HIV or HIV IC (β-globine gene).

HIV DNA Real-TM kit is a qualitative test based on the use of an endogenous control, the β-globin gene. The DNA target selected as an endogenous internal control is a human genome fragment that is present in sample in a sufficient quantity equivalent to that of cells in the sample. Internal Control (IC) serves as an amplification control for each individually processed specimen and to identify possible inhibition. IC is detected in a channel other than the HIV DNA. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification. The kit will allow the detection of 100 samples.

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (R-V1-D)

Part N° 2 – **"HIV DNA Real-TM":** Real Time amplification kit

- **PCR-mix-1-TM**, 8 x 0,24 mL.
- **PCR-mix-2-TM**, 8 x 0,2 mL.
- **TaqF Polymerase**, 8 x 0,02 mL
- **TE-buffer**, 8 x 0,07 mL
- **Pos HIV-IC C+***, 2 x 0,2 mL

Contains reagents sufficient for 120 reactions.

* *must be used in the isolation procedure as Positive Control of Extraction*

Module No.2: Real Time PCR kit (TR-V1-D)

Part N° 1 – **"Hemo-Sorb HIV":** Sample preparation kit

- **Hemolytic**, 2 x 100 ml;
- **Lysis Solution**, 30 ml (2x15ml);
- **Washing Solution 1**, 30 ml (2x15ml);
- **Washing Solution 2**, 100 ml (2x50ml);
- **Sorbent**, 2 x 1,25 ml
- **TE-buffer eluent**, 2 x 5,0 ml;

Contains reagents for 100 extractions.

Part N° 2 – **"HIV DNA Real-TM":** Real Time amplification kit

- **PCR-mix-1-TM**, 8 x 0,24 mL.
- **PCR-mix-2-TM**, 8 x 0,2 mL.
- **TaqF Polymerase**, 8 x 0,02 mL
- **TE-buffer**, 8 x 0,07 mL
- **Pos HIV-IC C+***, 2 x 0,2 mL

Contains reagents sufficient for 120 reactions.

* *must be used in the isolation procedure as Positive Control of Extraction*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g); Eppendorf 5415D or equivalent
- 60°C ± 2°C dry heat block
- Vortex mixer
- Pipettors (capacity 5-40 µl; 40-200 µl; 200-1000 µl) with aerosol barrier
- 1,5 ml polypropylene sterile tubes (Sarstedt, QSP, Eppendorf)
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: RT and amplification:

- Real Time Thermal cycler
- Tubes (0,2 ml)
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

STORAGE INSTRUCTIONS

Part N° 1 – **“Hemo-Sorb HIV”** must be stored at 2-8°C.

Part N° 2 – **“HIV DNA Real-TM ”** must be stored at -20°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

HIV DNA Real-TM Test 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

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.

PRODUCT USE LIMITATIONS

Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). 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

HIV DNA Real-TM can analyze DNA extracted with **Hemo-Sorb HIV** from:

- *Whole blood* collected blood in EDTA tubes;

Specimens can be stored at +2-8°C for no longer than 12 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.

DNA ISOLATION

The following kits are recommended:

- ⇨ **Hemo-Sorb HIV** (Sacace);
- ⇨ **SaMag Blood DNA Extraction kit** (Sacace, REF SM001).

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

SPECIMEN AND REAGENT PREPARATION

1. Prepare required quantity of 1,5 ml polypropylene tubes
2. Pipette 1 ml Hemolytic into 1.5-mL tubes with screw caps and add **250 µl** of **Samples** (newborns **100 µl**) to the appropriate tube. Close the tubes and vortex vigorously.
3. Incubate the tubes for 3 minutes, then stir and incubate for 3 minutes else.
4. Centrifuge all tubes at 8000 *g* for 2 minutes. Suck the supernatants out, using a separate tip for each tube.
5. Add **500 µl** of **Hemolytic** and vortex, incubate for 3 minutes.
6. Centrifuge all tubes at 8000 *g* for 2 minutes. Suck the supernatants out, using a separate tip for each tube
7. Repeat steps (5) and (6).

The dry pellet may be extracted immediately or stored at –70^{0}C until extraction

8. **Lysis Solution** and **Washing Solution** (in case of their storage at +2-8°C) should be warmed up to 60°C until disappearance of ice crystals.
9. Add to each tube **300 µl** of **Lysis Solution**
10. Prepare Controls as follows:
    - add **5 µl** of **TE-buffer** to the tube labeled C*neg;*
    - add **5 µl** of **Pos HIV-IC C+** to the tube labeled C*pos*
11. Vortex vigorously **Sorbent** and add **25 µl** to each tube.
12. Vortex for 5-7 sec and incubate all tubes for 6 min at room temperature. Vortex periodically.
13. Centrifuge all tubes for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
14. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously and centrifuge for 1 min at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
15. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously and centrifuge for 1 min at 10000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between tubes.
16. Repeat step 15.
17. Incubate all tubes with open cap for 10 min at 65°C.
16. Resuspend the pellet in **50 µl** of **TE-buffer eluent.** Incubate for 10 min at 65°C and vortex periodically.

Centrifuge the tubes for 2 min at maximum speed (12000-16000 g). The supernatant contains DNA ready for amplification. If amplification is not performed the same day of extraction store the DNA at 2-8°C for one week or at -16°C for one year.

PROTOCOL:

Note: Reaction Mix volume = 50 µl, the volume of DNA sample is 25 µl.
(The reaction mix for SmartCycler® instrument is 25 µl, the volume of DNA sample is 12.5 µl. The volumes of reagents for these formats are enclosed in brackets)

1. Thaw one set of reagents, vortex and centrifuge briefly the tubes. Prepare reaction tubes.
2. Prepare **Reaction Mix (15 reactions)**: add into the tube with **PCR-mix-1 160 µl** of **PCR-mix-2, 16 µl** of **TaqF Polymerase**. Vortex thoroughly and centrifuge briefly.
3. Add **25 µl (12,5 µl *for SmartCycler®*)** of **Reaction Mix** into each tube.
4. Add **25 µl (12,5 µl *for SmartCycler®*)** of **extracted DNA** sample to appropriate tube with Reaction Mix.
5. Prepare for each panel 3 controls:
   - add **25 µl (12,5 µl *for SmartCycler®*)** of **TE-buffer** to the tube labeled Negative Control;
   - add **25 µl (12,5 µl *for SmartCycler®*)** of **Pos HIV-IC C+** to the tube labeled Positive Control;

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**	20 s	5	**95**	20 s	5
	52	30 s		**52**	30 s	
	72	30 s		**72**	30 s	
3	**95**	20 s	40	**95**	20 s	42
	55	30 s *fluorescent signal detection*		**55**	40 s *fluorescent signal detection*	
	72	30 s		**72**	30 s	

[1] *For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, SaCycler-96™ (Sacace), CFX96™/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7300/7500/StepOne Real Time PCR (Applied Biosystems), SmartCycler (Cepheid)*

Fluorescence is detected at the 2nd step of Cycling 2 stage (55 °C) in FAM/Green and JOE/Yellow/Hex/Cy3 fluorescence channels.

HIV DNA is detected on the JOE(Yellow)/HEX/Cy3, *IC DNA* on the FAM (Green) 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*	*0.03*	*10 %*	*ON*
JOE/Yellow	*from 5 Fl to 10 Fl*	*0.03*	*5 %*	*ON*

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.

DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

The signal from the Internal Control amplification product is detected in the FAM fluorescence channel;

The signal from the *HIV* DNA amplification product is detected in the JOE channel.

Interpretation of results

The results are interpreted by the Instrument software by the crossing (or not-crossing) of the fluorescence curve with the threshold line and are shown as the presence (or absence) of Ct (threshold cycle) in the result grid.

The result of the analysis is considered reliable only if the results for Positive and Negative Controls of Amplification as well as Negative Control of Extraction are correct.

1. The sample is considered to be **positive** if its Ct value detected in the result grid in the channel for Positive Control is less than the specified boundary Ct value.

2. The sample is considered to be **negative** if its Ct value is not detected in the result grid in the channel for Positive Control (the fluorescence curve does not cross the threshold line) and if the Ct value determined in the results grid in the channel for IC does not exceed the specified boundary Ct value.

Boundary value of the cycle threshold, Ct

Sample	Stage for control	Ct boundary value Rotor Type Instrument		Ct boundary value Plate Type Instrument	
		JOE/Yellow/HEX	FAM/Green	JOE/Yellow/HEX	FAM/Green
NCA	Amplification	Absent	Absent	Absent	Absent
Pos HIV-IC C+	DNA extraction	<40	<20	<40	<24
Sample	DNA extraction	<40	<20	<40	<24

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific **HIV** primers and probes. The specificity of the kit **HIV DNA Real-TM** was 100%. The potential cross-reactivity of the kit **HIV DNA Real-TM** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **HIV DNA Real-TM** allows to detect **HIV** DNA in 100% of the tests with a sensitivity of not less than 250 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target region: pol gene

TROUBLESHOOTING

1. Ct value for Negative Control of extraction or amplification less than the boundary value.
    - Contamination of reagents or samples
        - ⇒ The result of analysis must be considered as invalid.
        - ⇒ Analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
2. 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.
        - ⇒ Storage conditions and expiration date of the reagents should be checked and then PCR should be repeated.
        - ⇒ Incorrect extraction procedure. Repeat analysis starting from DNA extraction stage.

KEY TO SYMBOLS USED

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

* SaCycler™ is a registered trademark of Sacace Biotechnologies
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* Rotor-Gene™ is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems
* SmartCycler® is a registered trademark of Cepheid

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