



# HCV/HBV/HIV1/HIV2 Real-TM

Handbook

Real Time PCR Kit

**REF** V62-100FRT

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**Σ** 100

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## NAME

### HCV/HBV/HIV1/HIV2 Real-TM

## INTRODUCTION

Transfusion-associated transmission risk of infectious diseases has been reported worldwide. Over the past two decades, a long series of specific and non-specific measures have been introduced into the screening of blood donations in order to reduce the residual risk of transmission of bloodborne viruses. The latest specific measure has been viral nucleic acid testing (NAT), introduced by the European plasma industry in 1995, and subsequently introduced for blood donations in many countries. NAT was implemented to reinforce the safety of the blood supply; it can detect acute viral infections during the 'window period', that are not detected by the serological screening methods.

It should be noted that, contrary to the classical serologic screening methods that are always used in single donation testing, current NAT procedures usually demand pooling of blood donation samples due to the format of the employed platforms. Today, NAT implementation for HCV, HBV and HIV is taken for granted in most high-income countries to ensure the maximal viral safety.

## INTENDED USE

The **HCV/HBV/HIV1/HIV2 Real-TM** kit is a qualitative in vitro test for comprehensive single-assay Real Time detection of Human Immunodeficiency Virus 1 Type (HIV1) RNA, Human Immunodeficiency Virus 2 Type (HIV2) RNA, hepatitis C Virus (HCV) RNA and hepatitis B Virus (HBV) DNA in human plasma with simultaneous detection of Internal Control (IC). This kit is intended for use as a donor screening test to detect HIV1 RNA, HIV2 RNA, HCV RNA and HBV DNA in plasma from individual donors which may be screened as individual samples or may be tested in pools comprised of equal aliquots. The recommended quantity of the samples in one pool must be not more than 5-10 (100-200 µl of the plasma for each sample).

## PRINCIPLE OF ASSAY

The **HCV/HBV/HIV1/HIV2 Real-TM** kit is a Real-Time test for the qualitative detection and differentiation of HIV1 RNA, HIV2 RNA, HCV RNA and HBV DNA in human plasma. RNA/DNA is extracted from plasma, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for HCV, HBV, HIV1, HIV2 and IC. IC is an Internal Control and represents recombinant RNA-containing-structure which carried through all steps of analysis from nucleic acid extraction to PCR amplification-detection. Internal Control (IC) serves as an extraction and amplification control for each individually processed specimen and to identify possible reaction inhibition.

- HCV cDNA is detected on the Fam (Green) channel,
- HIV1 cDNA on the Joe (Yellow)/HEX channel,
- HBV DNA on the Rox (Orange) channel,
- IC on the Cy5 (Red) channel
- HIV2 cDNA on the Cy5.5/Crimson/ Quasar 705 channel.

## MATERIALS PROVIDED

Part N° 1 – “Controls”

Part N° 2– “HCV/HBV/HIV1/HIV2 Real-TM”: RT Real Time;

Contents	Ref. V62-100FRT 100 reactions
<b>Part N°1 – “Controls”</b>	
Internal Control (IC) Rec <sup>1</sup>	2 x 0,5 ml
HCV/HBV/HIV1/HIV2 Rec Pos C+ <sup>2</sup>	2 x 0,2 ml
NCS (Neg. Control Sample) <sup>2</sup>	2 x 1,2 ml
<b>Part N°2–“HCV/HBV/HIV1/HIV2 Real-TM”</b>	
RT-PCR-mix-1-TM	2 x 0,3 ml
RT-PCR-mix-2-TM	2 x 0,3 ml
Hot Start Taq Polymerase	2 x 0,03 ml
M-MLV Revertase	2 x 0,015 ml
RT-G-mix-2	2 x 0,015 ml
TE-buffer	2 x 1,2 ml
Pos HCV/HBV/HIV1/HIV2 C+	2 x 0,1 ml

<sup>1</sup> must be used during the sample preparation procedure (see RNA/DNA isolation)

<sup>2</sup> must be used during the sample preparation procedure: add 100 µl of C- (Negative Control) to labeled Cneg; add 90 µl of C- (Negative Control) and 10 µl of HCV/HBV/HIV1/HIV2 Rec Pos C+ to the tube labeled Cpos

## MATERIALS REQUIRED BUT NOT PROVIDED

- RNA/DNA isolation kit (see RNA/DNA isolation)
- Microcentrifuge for “eppendorf” type tubes
- Vortex mixer
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator, Freezer
- Real Time PCR System
- Microcentrifuge for Smart Cycler’s reaction tubes
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Tube racks

## WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Use routine laboratory precautions. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas. Do not pipette by mouth.
3. Do not use a kit after its expiration date.
4. Do not mix reagents from different kits.
5. Dispose all specimens and unused reagents in accordance with local regulations.
6. Heparin has been shown to inhibit reaction. The use of heparinized specimens is not recommended.
7. Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
8. Once the reagents have been thawed, vortex and centrifuge briefly the tubes.
9. Prepare quickly the Reaction mix.
10. Specimens may be infectious. Use Universal Precautions when performing the assay.
11. Specimens and controls should be prepared in a laminar flow hood.
12. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
13. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant. Follow by wiping down the surface with 70% ethanol.
14. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
15. Material Safety Data Sheets (MSDS) are available on request.
16. Use of this product should be limited to personnel trained in the techniques of amplification.
17. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification Area. Do not return samples, equipment and reagents in the area where you performed previous step. Personnel should be using proper anti-contamination safeguards when moving between areas.

## STORAGE INSTRUCTIONS

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

Part N° 2 – “**HCV/HBV/HIV1/HIV2 Real-TM**” must be stored at -20°C.

The kit 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

**HCV/HBV/HIV1/HIV2 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.

## SAMPLE COLLECTION, STORAGE AND TRANSPORT

*Note: Handle all specimens as if they are potentially infectious agents.*

1. EDTA tubes may be used with the **HCV/HBV/HIV1/HIV2 Real-TM**. Follow sample tube manufacturer’s instructions.
2. Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for an additional 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
3. Do not freeze whole blood.
4. Specimens anti-coagulated with heparin are unsuitable for this test.
5. Thaw frozen specimens at room temperature before using.
6. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.
7. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

## RNA/DNA ISOLATION

The following isolation kits are recommended:

- ⇒ **Ribo-Sorb-100** (Sacace, [REF](#) K-2-1/100): sample volume 100 µl
- ⇒ **Ribo Virus 100**– spin column extraction kit (Sacace, [REF](#) K-2-C/100): sample volume 150 µl
- ⇒ **Magno-Virus** – Magnetic RNA/DNA extraction kit (Sacace [REF](#) K-2-16/200 or Sacace [REF](#) K-2-16/1000) sample volume 500 µl or 1000 µl from pool samples (up to 10 samples in a pool)
- ⇒ **SaMag Viral Nucleic Acids Extraction kit** (Sacace, [REF](#) SM003), sample volume 400 µl (up to 4 samples in a pool)

Please carry out the RNA extraction according to the manufacturer's instructions. Add 10 µl of Internal Control during the RNA/DNA isolation procedure directly to the sample/lysis mixture.

## REAGENT PREPARATION

**Note: Reaction Mix volume = 25 µl**

1. Thaw one set of reagents, vortex and centrifuge briefly the tubes.
2. Prepare reaction tubes.
3. Prepare **Reaction Mix** for 50 samples: add into the tube with **RT-PCR-mix-1-TM (0,3 ml) 300 µl of RT-PCR-mix-2-TM, 30 µl of TaqF Polymerase, 15 µl of M-MLV Revertase and 15 µl of RT-G-mix-2**. Vortex thoroughly and centrifuge briefly.

*(If it is necessary to test less than 50 samples add for each sample (N) in the new sterile tube 5\*N+1 µl of RT-PCR-mix-1-TM, 5\*N+1 µl of RT-PCR-mix-2-TM, 0,5 \*N+1 µl of TaqF Polymerase, 0,25\*N+1 µl of M-MML and 0,25\*N+1 µl of RT-G-mix-2)*

*Example of mix preparation:*

		Reagents volume for the quantity of tests (N+1)				
Reagents volume for 1 reaction, µl		5.00	5.00	0.50	0.25	0.25
Q.ty of clinical samples	Number of PCR reactions	RT-PCR-mix-1-TM	RT-PCR-mix-2-TM	TaqF Polymerase	RT-G-mix-2	M-MML
4	8	45	45	4.5	2	2
6	10	55	55	5.5	3	3
8	12	65	65	6.5	3.5	3.5
10	14	75	75	7.5	3.5	3.5
12	16	85	85	8.5	4.5	4.5
14	18	95	95	9.5	4.5	4.5
16	20	105	105	10.5	5.5	5.5
30	34	175	175	18	9	9
40	44	225	225	23	12	12
50	54	Whole tube	Whole tube	Whole tube	15	Whole tube

4. Add **10 µl of Reaction Mix** into each tube.
5. Add **15 µl of extracted RNA/DNA** sample to the appropriate tube with Reaction Mix and mix by pipetting avoiding air bubble.  
*If the Ribo-Sorb isolation kit is used as a RNA/DNA extraction kit, re-centrifuge all the tubes with extracted RNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction!*
6. Prepare for each panel 2 controls:
  - add **15 µl of TE-buffer** to the tube labeled Negative Control of Amplification;
  - add **15 µl of Pos HCV/HBV/HIV1/HIV2 C+** to the tube labeled Positive Control of Amplification;
7. Transfer the tubes in the thermalcycler.

## TEMPERATURE PROFILE

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

	Rotor type instruments <sup>1</sup>				Plate type or modular instruments <sup>2</sup>			
Stage	Temp, °C	Time	Fluorescence detection	Cycle repeats	Temp, °C	Time	Fluorescence detection	Cycle repeats
1	50	20 min	–	1	50	20 min	–	1
2	95	15 min	–	1	95	15 min	–	1
3	95	20 s	–	4	95	20 s	–	4
	46	40 s	–		46	40 s	–	
4	95	5 s	–	42	95	5 s	–	42
	60	40 s	–		60	40 s	–	
	45	30 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red Cy5.5/Crimson		45	40 s	FAM, HEX, ROX, Cy5, Cy5.5/Quasar 705	

<sup>1</sup> For example Rotor-Gene™ 6000/Q (Qiagen)

<sup>2</sup> For example, SaCycler-96 5x (Sacace), CFX™ (BioRad);

## RESULTS ANALYSIS

1. The results are interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line.

- HCV cDNA is detected on the Fam (Green) channel,
- HIV1 cDNA on the Joe (Yellow)/HEX channel,
- HBV DNA on the Rox (Orange) channel,
- IC on the Cy5 (Red) channel
- HIV2 cDNA on the Cy5.5/Crimson/ Quasar 705 channel.

Results are accepted as relevant if positive and negative controls of amplification along with negative and positive controls of extraction are passed (see table 1).

**Table 1. Results for controls**

Control	Stage for control	Fam (Green)	Joe (Yellow)/ HEX/Cy3	Rox/(Orange)/ TexasRed	Cy5 (Red)	Cy5.5/Crimson/ Quasar 705	Interpretation
NCS	RNA/DNA isolation	NEG	NEG	NEG	POS	NEG	Valid result
HCV/HBV/HIV1/HIV2 Rec Pos C+	RNA/DNA isolation	POS	POS	POS	POS	POS	Valid result
TE-buffer	Amplification	NEG	NEG	NEG	NEG	NEG	Valid result
Pos HCV/HBV/HIV1/HIV2 C+	Amplification	POS	POS	POS	POS	POS	Valid result

The Ct values for the controls are specific for every lot (reported on the HCV/HBV/HIV1/HIV2 Real-TM Data Card)

- ✓ The sample is considered to be positive for HCV if in the channel Fam (Green) the value of Ct is lower than 33.
- ✓ The sample is considered to be positive for HIV1 if in the channel Joe (Yellow)/HEX the value of Ct is lower than 33.
- ✓ The sample is considered to be positive for HBV if in the channel Rox (Orange) the value of Ct is lower than 33.
- ✓ The sample is considered to be positive for HIV2 if in the channel Cy5.5(Crimson)/Quasar705 the value of Ct is lower than 33.
- ✓ The sample is considered to be negative if in the channels Fam (Green), Joe (Yellow)/HEX, Rox (Orange), Cy5.5 (Crimson)/Quasar 705 the Ct value is not determined (the fluorescence curve does not cross the threshold line) and in the results table on the channel Cy5(Red) the Ct value is lower than 33.

## INSTRUMENT SETTINGS

### Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)

Channel	Calibrate/Gain Optimisation...	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	from 5 Fl to 10 Fl	0.05	5 %	on
JOE/Yellow	from 5 Fl to 10 Fl	0.05	3 %	on
Rox/Orange	from 5 Fl to 10 Fl	0.05	5 %	on
Cy5/Red	from 5 Fl to 10 Fl	0.03	10 %	on
Cy5.5/Crimson	from 5 Fl to 10 Fl	0.03	30 %	on

### Plate- or modular type instruments

For result analysis, set the threshold line at a level corresponding to 10–20% of the maximum fluorescence signal obtained for Pos C+ sample during the last amplification cycle.

## PERFORMANCE CHARACTERISTICS

### Analytical specificity

Analytical specificity of the primers and probes was validated with 90 negative samples. They did not generate any signal with the **HCV/HBV/HIV1/HIV2 Real-TM** kit primers and probes. The specificity of the kit **HCV/HBV/HIV1/HIV2 Real-TM** was 100%. The potential cross-reactivity of the kit **HCV/HBV/HIV1/HIV2 Real-TM** was also tested against a group control. It was not observed any cross-reactivity with any of the tested pathogens.

### Analytical sensitivity

The sensitivity of the **HCV/HBV/HIV1/HIV2 Real-TM** kit is:











- HCV RNA – 10 IU/ml
- HBV DNA – 5 IU/ml
- HIV1 RNA – 35 IU/ml
- HIV2 RNA – 60 copies/ml

The detection was carried out on the control standard and its dilutions by negative plasma using the “Magno-Virus” extraction kit (Sacace [REF](#) K-2-16/1000) starting from a sample volume of 1 ml.

## TROUBLESHOOTING

1. Weak (Ct > 33) signal of the IC (Cy5(Red) channel): retesting of the sample is required.
  - The PCR was inhibited.
    - ⇒ Make sure that you use a recommended RNA extraction method and follow the manufacturer’s instructions.
  - The reagents storage conditions didn’t comply with the instructions.
    - ⇒ Check the storage conditions
  - The PCR conditions didn’t comply with the instructions.
    - ⇒ Check the PCR conditions and for the IC detection select the fluorescence channel reported in the protocol.
  - The IC was not added to the sample during the pipetting of reagents.
    - ⇒ Make attention during the RNA extraction procedure.
2. Weak (Ct > 33) signal on one of the channels: retesting of the sample is required.
3. Any signal on Fam, Joe, Rox or Cy5.5 channel with Negative Control of extraction .
  - Contamination during RNA 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 among tubes.
    - ⇒ Repeat the RNA extraction with the new set of reagents.
4. Any signal with Negative PCR Control.
  - 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 controls at the end.
    - ⇒ Repeat the PCR preparation with the new set of reagents.

## EXPLANATION OF SYMBOLS

	List Number		Caution!
	Lot Number		Contains sufficient for <n> tests
	Expiration Date		Version
	Store at	<b>NCA</b>	Negative Control of Amplification
	Manufacturer	<b>NCE</b>	Negative control of Extraction
	Consult instructions for use	<b>C+</b>	Positive Control of Amplification
<b>IC</b>	Internal Control		For Research Use Only

\*SaCycler-96™ is a registered trademark of Sacace Biotechnologies

\*CFX™ is trademark of Bio-Rad Laboratories

\*Rotor-Gene™ Technology is a registered trademark of Qiagen



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