Sacace BIOTECHNOLOGIES

IVD For *in Vitro* Diagnostic Use

CE

⚠ **NEW FORMAT**

Rotavirus/Norovirus/Astrovirus Real-TM

for use with RotorGene™ 3000/6000 (Corbett Research), SmartCycler® (Cepheid), iQ iCycler™ and iQ5™ (Biorad), Applied Biosystems® 7300/7500 Real Time PCR Systems (Applera), MX3000P® and MX3005P® (Stratagene)

REF **V40-50FRT**

VER **20.01.2010**

Σ **50**

NAME

Rotavirus/Norovirus/Astrovirus Real-TM

INTENDED USE

Kit **Rotavirus/Norovirus/Astrovirus Real-TM** is a Real-Time test for the qualitative detection and differentiation of *Rotavirus A, Norovirus 2 genotype, Astrovirus* in the biological materials and in the environment. RNA is extracted from specimens, amplified using RT-amplification and detected using fluorescent reporter dye probes specific for Rotavirus/Norovirus/Astrovirus RNA and IC (Internal Control).

PRINCIPLE OF ASSAY

Rotavirus/Norovirus/Astrovirus Real-TM Test is based on three major processes: isolation of *virus* RNA from specimens, one-step reverse transcription of the RNA and Real Time amplification of the cDNA. *Rotavirus/Norovirus/Astrovirus* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers and detection via fluorescent dyes. These dyes are linked with probes of oligonucleotides which bind specifically to the amplified product. The real-time PCR monitoring of fluorescence intensities allows the accumulating product detection without reopening of reaction tubes after the PCR run. **Rotavirus/Norovirus/Astrovirus Real-TM** PCR kit is a qualitative test which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the process of each individual sample extraction and serves also to identify possible reaction inhibition.

MATERIALS PROVIDED

Part N° 1 – **"Rotavirus/Norovirus/Astrovirus Real-TM":** RT Real Time kit;
Part N° 2 – **"Controls"**

Part N° 1 – **"Rotavirus/Norovirus/Astrovirus Real-TM":**

- **PCR-mix-1 *Rotavirus* / *Astrovirus***, 0,6 ml;
- **PCR-mix-1 *Norovirus* / *IC*** 0,6 ml;
- **RT-PCR-mix-2**, 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;

Contains reagents for 55 reactions

Part N° 2 – **"Controls"**

- **Negative Control C-**, 1,6 ml;*
- **Internal Control (IC RNA)**, 5 x 0,12 ml.**
- **Pos cDNA *Rotavirus*/*Astrovirus* C+,** 0,1 ml;
- **Pos cDNA *Norovirus* 2 / *Internal Control (IC)* C+,** 0,1 ml;
- **DNA-buffer**, 0,5 ml;

** must be used during the sample preparation procedure: add 100 µl of C– (Negative Control) to labeled Cneg*
***add 10 µl of Internal Control to all samples during the RNA isolation procedure directly to the sample/lysis mixture*

MATERIALS REQUIRED BUT NOT PROVIDED

- Real Time Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
7. 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.
8. Material Safety Data Sheets (MSDS) are available on request.
9. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
10. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Part N° 1 – **"Rotavirus/Norovirus/Astrovirus Real-TM"** must be stored at -20°C.
Part N° 2 – **"Controls"** must be stored at 2-8°C.

STABILITY

Rotavirus/Norovirus/Astrovirus 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.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Rotavirus/Norovirus/Astrovirus Real-TM can analyze RNA extracted with **Ribo-Sorb** from:

- *water:* centrifuge 10-20 ml for 10 min at maximum speed. Discard the supernatant and leave about 100 µl of solution for DNA extraction;
- *whole blood* collected in EDTA tubes*;*
- *feces:*
    - Prepare 20% feces suspension by adding in 5 ml tube of 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces. Vortex to get the homogeneous suspension and centrifuge for 5 min to 7000-12000g and using a micropipette with a plugged aerosol barrier tip transfer in a new sterile 1,5 ml tube 50 µl of the bacterial fraction (white-yellowish line between the sediment and the supernatant)

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.

RNA ISOLATION

The following isolation kit is recommended:

⇒ **Ribo-Sorb-** (Sacace, REF K-2-1)

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

RT AND AMPLIFICATION

Total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.

1 Prepare required quantity of reaction tubes (2 tubes for each sample + Controls)

2 Prepare the reaction mix for required number of samples.

3 For N reactions mix for each PCR-Mix-1 in a new tube:

10*(N+1) µl of RT-PCR-mix-1 *Rotavirus* / *Astrovirus* (or *Norovirus* / *IC)*
5.0*(N+1) µl of RT-PCR-mix-2
0.5*(N+1) µl of Polymerase
0.25*(N+1) µl of RT-G-mix-2
0.25*(N+1) µl of MMlv

4 Vortex the tube, then centrifuge shortly. Add **15 µl** of prepared reaction mix into each appropriate tube.

5 Using tips with aerosol filter add **10 µl** of RNA samples obtained at the stage of RNA isolation and mix carefully by pipetting**.**
N.B. If the Ribo-Sorb isolation kit is used as a RNA 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 3 controls:

- add **10 µl** of **DNA-buffer** to the tube labeled Amplification Negative Control;
- add **10 µl** of **Pos cDNA *Rotavirus*/*Astrovirus*** to the tube with **PCR-mix-1 *Rotavirus* / *Astrovirus;***
- add **10 µl** of **Pos cDNA *Norovirus* 2 / *IC* C+** to the tube with **PCR-mix- *Norovirus* / IC**

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

	Rotor type instruments[1]				*Plate type or modular instruments*[2]			
Stage	*Temp, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*	*Temp,°C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	50	30 min	–	1	50	30 min	–	1
Hold	95	15 min	–	1	95	15 min	–	1
Cycling 2	95	10 s	–	45	95	10 s	–	45
	60	25 s	FAM(Green), JOE(Yellow)		60	30 s	FAM, JOE/HEX/Cy3	
	72	10 s	–		72	10 s	–	

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

[2] For example, iQ5™/iQ iCycler™ (BioRad, USA); Mx3000P/Mx3005P™ (Stratagene, USA), Applied Biosystems® 7300/7500 Real Time PCR (Applera), SmartCycler® (Cepheid)

RESULTS ANALYSIS

1. The results are interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line.
   - Internal Control (IC) is detected on the FAM (Green) channel and *Norovirus* on the JOE (Yellow)/HEX/Cy3 channel with PCR-mix- *Norovirus* / IC;
   - *Rotavirus A* is detected on the FAM (Green) channel and *Astrovirus* on the JOE (Yellow)/HEX/Cy3 channel with PCR-mix-1 *Rotavirus* / *Astrovirus;*
2. The sample is considered to be positive if the value of **Ct** is different from zero (Ct < 33)
3. The sample is considered to be negative if the result is positive only on the channel Fam with PCR-mix- *Norovirus* / IC STI-87-rec and the Ct value is lower than 33.

EXPLANATION OF SYMBOLS

Catalogue Number

For *in Vitro* Diagnostic Use

Lot Number


Expiration Date

Contains reagents


Caution!

Version


Manufacturer


Temperature limitation

*iCycler™ and iQ5™ are trademarks of Bio-Rad Laboratories
* Rotor-Gene™ Technology is a registered trademark of Corbett Research
*MX3000P® and MX3005P® are trademarks of Stratagene
*Applied Biosystems® is trademarks of Applera Corporation
* SmartCycler® is a registered trademark of Cepheid

Sacace Biotechnologies Srl
44 Scalabrini str., 22100 Como, Italy

*PCR: The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-La Roche and applicable in certain countries. Sacace does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this kit is recommended for persons that either have a license to perform PCR or are not required to obtain a license