Enterovirus Real-TM
Real Time Kit for use with Rotor-Gene™ 2000/3000/6000 (Corbett Research)

Key to symbols used

- **REF** List Number
- **IVD** For in Vitro Diagnostic Use
- **LOT** Lot Number
- **VER** Version
- **RC** Store at 2-8°C/-20°C
- **Caution!**
- **Consult instructions for use**
- **Contains reagents**
- **Manufacturer**

**NAME**
Enterovirus Real-TM

**INTRODUCTION**

**Enteroviruses** are a genus of (+)ssRNA viruses associated with several human and mammalian diseases. Serologic studies have distinguished 66 human enterovirus serotypes on the basis of antibody neutralization tests. Additional antigenic variants have been defined within several of the serotypes on the basis of reduced or nonreciprocal cross-neutralization between variant strains. On the basis of their pathogenesis in humans and animals, the enteroviruses were originally classified into four groups, polioviruses, Coxsackie A viruses (CA), Coxsackie B viruses (CB), and echoviruses, but it was quickly realized that there were significant overlaps in the biological properties of viruses in the different groups. Enteroviruses affect millions of people worldwide each year, and are often found in the respiratory secretions (e.g., saliva, sputum, or nasal mucus) and stool of an infected person. Historically, poliomyelitis was the most significant disease caused by an enterovirus, Poliovirus. There are 62 non-polio enteroviruses that can cause disease in humans: 23 Coxsackie A viruses, 6 Coxsackie B viruses, 28 echoviruses, and 5 other enteroviruses. Poliovirus, as well as coxsackie and echovirus are spread through the fecal-oral route. Infection can result in a wide variety of symptoms ranging from mild respiratory illness (common cold), hand, foot and mouth disease, acute hemorrhagic conjunctivitis, aseptic meningitis, myocarditis, severe neonatal sepsis-like disease, and acute flaccid paralysis.
INTENDED USE
Kit Enterovirus Real-TM is a Real-Time test for the qualitative detection of Enterovirus RNA 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 Enterovirus RNA and IC (Internal Control) in the Rotor-Gene™ 2000/3000/6000 (Corbett Research).

PRINCIPLE OF ASSAY
Kit Enterovirus Real-TM is based on three major processes: isolation of RNA from specimens, reverse transcription of the RNA and Real Time amplification

MATERIALS PROVIDED
“Reverta-L”:
• RT-G-mix-1, 5 x 0,01 ml;
• RT-mix, 5 x 0,125 ml;
• Reverse transcriptase (M-MLV), 0,03 ml;
• TE-buffer, 1,2 ml.
Contains reagents for 60 tests.

“Enterovirus Real-TM”:
• PCR-mix-1 Enterovirus 55 ready-to-use single-dose test tubes;
• PCR-mix-2-Flu, 0,77 ml;
• Internal Control RNA (IC RNA)**, 5 x 0,12 ml;
• Internal Control cDNA (IC cDNA), 0,1 ml;
• Negative Control 1,2 ml**;
• Positive Control Enterovirus cDNA C+, 0,1 ml;
• DNA-buffer, 0,5 ml;
Contains reagents for 55 reactions
* must be used in the isolation procedure as Negative Control of Extraction.
** add 10 µl of Internal Control RNA during the RNA purification procedure directly to the sample/lysis mixture

MATERIALS REQUIRED BUT NOT PROVIDED
• Real Time Thermal cycler
• 60°C ± 2°C dry heat block
• Reaction tubes
• Workstation
• Pipettes (adjustable)
• Sterile pipette tips with filters
• Desktop centrifuge with rotor for 1,5/2,0 ml tubes
• Vortex mixer
• Freezer, refrigerator

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. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. 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.
9. Material Safety Data Sheets (MSDS) are available on request.
10. This kit is designed for use with “Ribo-Sorb” extraction kit. It is the user’s responsibility if other kits than “Ribo-Sorb” are used to perform this RNA extraction.
11. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
12. 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
“Reverta-L” must be stored at -20°C. “Enterovirus Real-TM” must be stored at 2-8°C. Kit can be shipped at 2-8°C but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY
Enterovirus Real-TM Test is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT
Enterovirus Real-TM can analyze RNA extracted from:
- liquor (ready for extraction)- 0,1 ml;
- 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 10-20% feces suspension, for instance adding 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces in 5 ml tube (the same can be done in 2,0 ml tube). The DNA/RNA purification must be done immediately, if it is not possible add 20% Glycerol sterile solution (cryoprotective agent that provides intracellular and extracellular protection against freezing) and store at -20°C.
  - Vortex to get an homogeneous suspension and centrifuge for 5 min to 7000-12000g. Use the supernatant for the extraction of the viral DNA/RNA.
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 kits are recommended:
⇒ Ribo Virus spin column extraction kit (Sacace, REF K-2-C)
⇒ Ribo-Sorb- (Sacace, REF K-2-I)
Please carry out the RNA extraction according to the manufacturer’s instructions. Add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture.

RT AND AMPLIFICATION
Reverse Transcription:
1) Prepare Reaction Mix: for 12 reactions, add 5,0 µl RT-G-mix-1 into the tube containing RT-mix and vortex for at least 5-10 seconds, centrifuge briefly. This mix is stable for 1 month at -20°C. Add 6 µl M-MLV into the tube with Reagent Mix, mix by pipetting, vortex for 3 sec, centrifuge for 5-7 sec (must be used immediately after the preparation).
(If it is necessary to test less than 12 samples add for each sample (N) in the new sterile tube 10*N µl of RT-G-mix-1 with RT-mix and 0,5*N µl of M-MLV).
2) Add 10 of Reaction Mix into each sample tube.
3) Pipette 10 µl RNA samples to the appropriate tube. (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). Carefully mix by pipetting.
4) Place tubes into thermalcycler and incubate at 37°C for 30 minutes.
5) Dilute 1: 2 each obtained cDNA sample with TE-buffer (add 20 µl TE-buffer to each tube).
cDNA specimens could be stored at -20°C for a week or at -70°C during a year.

PROTOCOL (Reaction volume 25 µl):
1. Prepare required quantity of PCR-mix-1 Enterovirus tubes for samples and controls (3 tubes).
2. Add 7 µl of PCR-mix-2 Flu into each tube.
3. Add 10 µl of cDNA to appropriate tube.
4. Prepare for each panel the following controls:
   • add 10 µl of DNA-buffer to the tube labeled PCR Negative Control;
   • add 10 µl of Enterovirus cDNA C+ to the tube labeled PCR Pos Control
   • add 10 µl of IC cDNA to the tube labeled IC Pos Control
Real Time Amplification with Rotor-Gene 2000/3000/6000

**Important:** For the Rotor-Gene 6000 must be used software 1.7 Build 67 or updated version (for software information contact info@sacace.com).

2. Click **New** in the main menu, select **Dual Labeled Probe. Click New**
3. Select Rotor Type **36-Well Rotor and No Domed 0.2 ml Tubes**
4. Reaction volume 25 µl
5. Program Rotor-Gene 2000/3000/6000 as follows:
   1. Hold 95 °С - 15 min
   2. Cycling 95 °С - 10 s
      54 °С - 20 s – detection
      72 °С – 10 s
   Cycle repeats – 45 times.

*fluorescence detection on the channels Fam (Green), and Joe (Yellow) on the 2-nd pass

Make the adjustment of the fluorescence channel sensitivity: **Channel Setup → Calibrate (Gain Optimisation for RG6000) → Perform Calibration (Optimisation) Before 1-st Acquisition.** In the window “Channel Settings” indicate **Min Reading 5, Max Reading 10** for both channels. In the column Tube position program position of the tubes in the carousel of the Rotor-Gene 2000/3000/6000 (the 1st position must contains reaction tube with reagents). Close the window **Auto Gain Calibration Setup.** Make sure that for RG6000 the window “15 µL oil layer volume” is selected.

RESULTS ANALYSIS:
1. Press **Analysis** then select button **Quantitation** and press **Show.** Select in the window **Quantitation Analysis Threshold 0,05, Dynamic Tube** for Fam (Green) channel and **Quantitation Analysis Threshold 0,1, Dynamic Tube** for Joe (Yellow) channel.
2. The results are interpreted with the software of Rotor-Gene 2000/3000/6000 through the presence of crossing of fluorescence curve with the threshold line:
   - Internal Control (IC) is detected on the FAM (Green) channel and Enterovirus cDNA is detected on the JOE (Yellow).
3. The sample is considered to be positive if the value of Ct on the Joe (Yellow) is different from zero (Ct < 33)
4. The sample is considered to be negative if in the channel Joe (Yellow) the Ct value is not determined (the fluorescence curve does not cross the threshold line) and in the results table on the channel Fam (Green) the Ct value is lower than 33.

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 Enterovirus primers and probes. The specificity of the kit Enterovirus Real-TM was 100%. The potential cross-reactivity of the kit Enterovirus Real-TM was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity
The kit Enterovirus Real-TM allows to detect Enterovirus RNA in 100% of the tests with a sensitivity of not less than 1000 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

**Target region:** 5’UTR