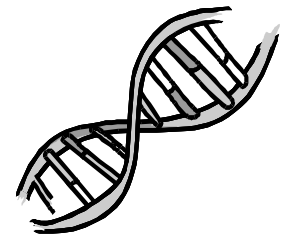




MutaREAL[®] Enterovirus

real time RT-PCR Kit



Screening assay for the *real time* detection of human enteroviruses (Polio-, Coxsackie A-, Coxsackie B- and Echoviruses) in *real time* PCR capillary systems (e.g. LightCycler[®], Roche).

REF KV2900224 

REF KV2900296 



For in vitro Diagnostic use only



Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
www.immundiagnostik.com
info@immundiagnostik.com

Tel.: +49 (0)6251/ 701900
 Fax: +49 (0)6251/ 849430



1. INTENDED USE

The **MutaREAL[®] Enterovirus** *real time* RT-PCR Kit is a quantitative screening assay for the detection of Enteroviruses (Polio-, Coxsackie A-, Coxsackie B- and Echoviruses) in clinical specimens (whole blood, plasma, respiratory samples, CSF [cerebrospinal fluid] etc.) in *real time* PCR capillary systems (e.g. LightCycler[®], Roche).

2. INTRODUCTION

Human enteroviruses (until now there are 66 serotypes known, belonging to the family of Picornaviridae) are ubiquitous pathogens with a high incidence worldwide (about 500 million infections/ year).

Enteroviruses may cause life-threatening infections, especially among children. Diseases such as myocarditis, paralysis, multiple organ failure, meningitis and encephalitis may be associated with enterovirus infections.

Human Enteroviruses are small, non-enveloped viruses with a ss-RNA genome of 6-7 kb. Thus far, there is no effective antiviral chemotherapy available. Route of transmission is fecal-oral, but the viruses can also be transmitted via contaminated food, knives and forks and others and are highly contagious.

3. PRINCIPLE OF THE TEST

The **MutaREAL[®] Enterovirus** *real time* RT-PCR Kit contains specific primers, TaqMan probes and additional material for the detection of Enteroviruses in clinical samples by use of the LightCycler[®] instrument (Roche). Target sequence for the detection is within the 5'-untranslated region (5'-UTR) of the genome.

The assay uses a reverse transcriptase to convert viral RNA into cDNA and a thermostable DNA polymerase to amplify a specific gene fragment by means of PCR (polymerase chain reaction). Furthermore in the same step proof of specificity is achieved *real time* by hybridization of the amplicon with a specific hybridization probe (oligonucleotides labelled with a fluorophore and a quencher molecule) after which as consequence, fluorescence is emitted and measured by the LightCycler[®]'s optical unit. Clinical samples, such as whole blood, plasma, respiratory samples, CSF (cerebrospinal fluid) etc. can be used. The detection of amplified Enterovirus RNA is performed in fluorimeter channel F1.

By the use of an internal control that is included in each reaction and that is co-amplified and detected, a possible inhibition of the reaction can be determined. The detection of amplified internal control is performed in channel F3.

4. KIT CONTENT

Each kit contains enough reagents to perform 24 respectively 96 tests. Each kit also contains a package insert.

Ref.	Type of reagent	Presentation	24	Presentation	96	Cap color
A1	Enzyme-Mix	1 vial,	30 µl	1 vial,	90 µl	blue
A2	Primer-/ Probe-Mix	1 vial,	400 µl	2 vials,	750 µl	yellow
A3	Positive control	1 vial,	20 µl	1 vials,	50 µl	red
A4	Negative control	1 vial,	200 µl	1 vial,	200 µl	green

5. TEST PERFORMANCE

Required materials - provided:

- PCR reagents
- Package insert

Required materials - not provided:

- *Real time* PCR system (e. g. LightCycler® instrument, Roche)
- *Real time* PCR reaction tubes (e. g. LightCycler® capillaries, Roche)
- Table centrifuge (e. g. LightCycler® capillary centrifuge, Roche)
- Cryo-container (e. g. LightCycler® cooling block, Roche)
- Color compensation kit for the used *real time* PCR system
- RNA extraction kit
- Pipets (0.5 µl – 200 µl)
- sterile filter tips for micro pipets
- sterile microtubes

6. STORAGE AND HANDLING

- All reagents (A1 to A4) should be stored at -20°C.
- All reagents can be used until the expiration date printed on the labels.
- Do not freeze and thaw the reagents A1, A2 and A3 several times.
- Use LightCycler® Cooling Block (Roche) or cool all reagents during the working steps.
- Primer-/ Probe-Mix (A2) should be stored in the dark.

7. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials.
- This assay needs to be run according to GLP (Good Laboratory Practice).
- Do not use the kit after its expiration date.

AMPLIFICATION

The PCR technology is utmost sensitive. Thus, amplification of a single molecule generates millions of identical copies. These copies may evade through aerosols and sit on surfaces. In order to avoid contamination of samples with DNA which previously was amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Set up two separate working areas:

- 1) Isolation of the RNA
- 2) Amplification/ detection of amplification products

Pipets, vials and other working materials should not circulate among working units!

- Use always sterile pipette tips with filters
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipettes and the laboratory benches with decontaminant
- Avoid aerosols

8. PROCEDURE

The complete procedure is separated in three steps:

- A) RNA extraction
- B) Reverse transcription of the RNA and following amplification and combined detection of RNA templates by the TaqMan probes (LightCycler[®] PCR)
- C) Interpretation of the results using the LightCycler[®] software

A) RNA EXTRACTION

- 1) RNA Extraction (by use of a commercial available RNA isolation kit):
Extract viral RNA from clinical samples by use of a commercial RNA isolation kit (suited for whole blood, plasma, respiratory samples, CSF [cerebrospinal fluid] etc.) according to the manufacturer's instructions.
- 2) If the LightCycler[®] PCR is not performed immediately, store extracted RNA at -20°C.

B) *Real time* Enterovirus RT-PCR PROTOCOL

Please read carefully the manual before starting the procedure! The Master Mix volume for the respective number of samples and controls should be pipetted as follows:

- 1) The Enzyme Mix volume per reaction and sample (N) should be multiplied with the number of samples to be performed, including controls A3 and A4. For reasons of unprecise pipetting, add an extra (virtual) sample. Proceed in the same manner with all additional reagents!

Cool all reagents during the working steps!

Reaction Volume		Master Mix volume
0.8 µl	Enzyme Mix (A1)	0.8 µl x (N+1)
14.2 µl	Primer-/ Probe Mix (A2)	14.2 µl x (N+1)

- 2) Mix gently (**do NOT vortex!**) the following reagents in a sterile tube: Enzyme Mix (A1) and Primer-/ Probe-Mix (A2). This mixture is the Master Mix. Spin down briefly in a table centrifuge.
- 3) Pipet **15 µl** of Master Mix using micropipets with sterile filter tips in each of the LightCycler[®] capillaries. Add **5 µl** of the RNA sample or positive and negative controls (A3 and A4) to each of these capillaries (it is recommended to pipet the negative control first to avoid contamination). Immediately lock the capillaries to avoid contamination.

Spin down briefly (in a LightCycler[®] capillary centrifuge).

Perform the following LightCycler[®] **RT-PCR protocol**:

45°C for **30 min**

95°C for **2 min**

95°C for **0 sec**

55°C for **30 sec**

72°C for **20 sec**

45 cycles

ramping time: 20°C/sec – aqu. mode here: SINGLE

40°C for **30 sec**

D) RT- PCR ANALYSIS AND INTERPRETATION OF RESULTS

- 1) Perform the LightCycler® PCR.
- 2) Switch on the Colour Compensation Filter (required because of the simultaneous use of two differently labelled TaqMan probes) by activating the field *Choose CCC File*.
- 3) The result for Enterovirus amplification is shown in channel F1, the result for the internal control is shown in channel F3.
- 4) Following results can arise:

- A signal is detected in channel F1.

The result is positive: The sample contains *Enterovirus* RNA.

In this case, the detection of a signal in channel F3 is inessential, as high concentrations of Enterovirus RNA can lead to a reduced or absent fluorescence signal of the internal control in channel F3 (competition).

- In channel F1 no signal is detected, only in channel F3 (signal of the internal control).

The sample does not contain any *Enterovirus* RNA.

The detected signal of the internal control excludes the possibility of an RT-PCR inhibition.

- Neither in channel F1 nor in channel F3 is a signal detected.

A diagnostic statement can not be made.

Inhibition of the RT-PCR reaction.

