



Enterovirus real time RT-PCR Kit (TaqMan)

Cat. No: 945200
For in vitro diagnostic use

with Extraction Control
96 Determinations

INTENDED USE

The AnDiaTec® Enterovirus real time RT-PCR Kit (TaqMan) is a screening assay for the detection of Enteroviruses (Coxsackie A, Coxsackie B and Echovirus) in clinical specimens (whole blood, plasma, respiratory samples, CSF (cerebrospinal fluid) etc.) using real time PCR microtiter plate systems (e.g. Applied Biosystems, Roche LC480, Stratagene, Qiagen/Corbett Research).

SUMMARY

Human Enteroviruses (until now 66 serotypes are known, belonging to the family of Picornaviridae) are ubiquitous pathogens with a high incidence worldwide (ca. 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 are no effective antiviral chemotherapeutics available. Route of transmission is fecal-oral, but the viruses can also be transmitted via contaminated food, knives, forks and others, and are highly contagious.

PRINCIPLE OF THE TEST

The AnDiaTec® Enterovirus real time RT-PCR Kit (TaqMan) contains specific primers, probes and additional material for the detection of Enterovirus in clinical samples.

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, proof of specificity is achieved in real time in the same step by hybridization of the amplicon with a specific probe. As consequence, fluorescence is emitted and measured by the real time PCR machine's optical unit (e.g. ABI PRISM SDS, Roche LC480, Stratagene MxPro, Qiagen/Corbett Research Rotogene 3000/6000 software). Clinical samples, such as whole blood, plasma, respiratory samples, CSF (cerebrospinal fluid) etc. can be used. Enterovirus specific amplification is measured by FAM fluorescence.

Furthermore, by the use of an Extraction 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 Extraction Control is performed in channel VIC/HEX.

REAGENTS PROVIDED

Each kit contains enough reagents to perform 96 test. Each kit also contains a package insert.

For real time PCR

Ref.	Type of Reagent	Presentation	Cap Color
A1	Enzyme-Mix	1 vial, 90µl	blue
A2	Primer and Probe-Mix	1 vial, 1.4ml	yellow
A3	Positive Control	1 vial, 50µl	red
A4	Negative Control	1 vial, 200µl	green
A5	Extraction Control	1 vial, 1.0ml	white

STORAGE AND HANDLING

All reagents (A1 to A5) should be stored at -20°C. All reagents can be used until the expiration date printed on the labels. Avoid multiple freezing and thawing cycles of reagents (<2). If used sporadically, prepare aliquots of the reagents. Cool all reagents during the working steps. Avoid exposure of A2 to light.

ASSAY PROCEDURE

Required material provided:

- PCR reagents
- Package insert

Required material not provided:

1. ABI system or a comparable instrument (e.g. Roche LC480, Stratagene, Qiagen/Corbett Research)
2. TC II reaction plate, 96 wells (Applied Biosystems) or comparable optical microtiter plates or optical reaction tube
3. Optical adhesive covers (Applied Biosystems) or comparable covers
4. RNA extraction kit
5. Pipets (0.5µl - 200µl) with sterile filter tips
6. Sterile microtubes

WARNINGS AND PRECAUTIONS

- This assay needs to be carried out by skilled personell!
- Clinical samples should be regarded as potentially infectious materials.
- This assay needs to be run according to GLP (Good Laboratory Practice).

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 RNA which was previously amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Pipets, vials and other working materials should not circulate among working units!

- Do not use the kit after its expiration date
- Set up (if possible) two separate working areas:
 1. Isolation of the RNA
 2. Amplification/ detection of amplification products
- Always use sterile pipet tips with filter
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipets and the laboratory benches with decontaminant
- Avoid aerosols

PROCEDURE

The complete procedure is separated into three steps:

1. Sample preparation and RNA extraction.
2. Amplification and combined detection of RNA fragments using reverse transcriptase and probes.
3. Interpretation of the results using the PCR machine's software.

1. RNA EXTRACTION

1.1 Pipet 10µl of the Extraction Control (A5) into 200µl sample material (whole blood, plasma, respiratory samples, CSF (cerebrospinal fluid) etc). Mix well!

1.2 Extract genomic RNA by use of a commercial RNA isolation kit from prepared samples according to the manufacturer's instructions.

Please note: 10µl of Extraction Control (A5) is optimized for 50µl of elution volume. If a larger elution volume is required, please adjust Extraction Control volume accordingly.

1.2 If real time RT-PCR is not performed immediately, store extracted RNA at -20°C.

2. Enterovirus real time RT-PCR Protocol

Please read carefully the manufacturer's instructions before starting the procedure! Each assay should include a Negative and Positive Control. Use filter tips for all pipetting.

2.1 The Enzyme-Mix volume per reaction/ sample should be multiplied by the number of samples (n), including the 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
0.8µl Enzyme-Mix (A1)	0.8µl x (n + 1)
14.2µl Primer and Probe-Mix (A2)	14.2µl x (n + 1)

Mix gently (do NOT vortex) the following reagents in a sterile tube: Enzyme-Mix (A1) and Primer and Probe-Mix (A2). This mixture is the Master-Mix. Spin down briefly in a table centrifuge.

2.2 Pipet 15µl of the Master-Mix per well of a 96 wells optical microtiter plate. The number of wells used is calculated from the number of samples plus one Positive and one Negative Control.

Add 5µl of sample RNA or Positive or Negative Control per well. Pipet the Negative Control first. To avoid contamination, it is advisable to cover the wells containing the Negative Control with an adhesive seal while pipetting the Positive Control and sample RNA. Remove this adhesive seal after preparing the wells.

2.3 Cover 96 wells optical microtiter plate with optical adhesive cover.

2.4 Run the RT-PCR using the following temperature protocol:

45°C for 30 min

95°C for 2 min

45 cycles of:

95°C for 0 sec

55°C for 30 sec measure fluorescence at the end of this step

72°C for 20 sec

3. RT-PCR ANALYSIS AND INTERPRETATION OF THE RESULTS

Enterovirus specific amplification is detected by FAM fluorescence. The Extraction Control is measured by VIC/ HEX fluorescence. Use following settings to define a reporter and quencher with the real time PCR software:

Detection	Reporter	Quencher
Enterovirus RNA	FAM	none
Extraction Control	VIC/ HEX	none
Reference Dye		none

NOTE: The Positive and Negative Control **do not** show amplification in channel F3, as no Extraction Control is included. Following results can arise:

3.1 FAM fluorescence is detected:

The result is positive.

The sample contains Enterovirus RNA.

The occurrence of VIC/HEX fluorescence is inessential as high concentrations of Enterovirus RNA may reduce or even inhibit the amplification of the Extraction Control.

3.2 No FAM, but VIC/ HEX fluorescence is detected:

The result is negative.

The sample does not contain Enterovirus RNA.

The detected signal of the Extraction Control excludes the possibility of an inhibition of the RT-PCR.

3.3 Neither FAM nor VIC/ HEX fluorescence is detected:

A diagnostic statement cannot be made.

An inhibition of the RT-PCR occurred.

RELATED PRODUCTS

Enterovirus real time RT-PCR Kit (TaqMan), Cat. No. 945100:

Kit for the detection of Enterovirus (Coxsackie A, Coxsackie B and Echovirus) in PCR microtiter plate systems (e.g. Applied Biosystems, Roche LC480, Stratagene, Qiagen/Corbett Research) with an internal Inhibition Control included within the Primer and Probe-Mix.

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