



EliGene® Adenovirus RT

REF 90036-RT (for 50 samples)

Kit components:

5 x 200 µl Adenovirus RT Mix
5 x 200 µl IC DNA
2 x 50 µl PC DNA Adenovirus
Instruction for Use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® Adenovirus RT kit is qualitative in vitro diagnostic device intended for the detection of adenovirus in clinical material.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and TaqMan labeled probes (FAM and YakimaYellow) for the detection of ADV DNA and internal control are used.

Introduction

Adenoviruses represent the largest non-enveloped viruses. They are able to be transported through the endosome (i.e. envelope fusion is not necessary). They are about 70 to 90 nm in size, nonenveloped icosahedral viruses which are made from up to 252 capsomeres containing double-stranded DNA. The replication cycle of adenovirus takes about 36 hours and newly produced virions are released from the cell as a result of virally induced cell lysis.

Infection is usually transmitted by droplets of respiratory or ocular secretions, by alimentary transfer or sexual intercourse and by the contact with contaminated things or water (virus is able to live out of body at temperature 20°C and lower for a few weeks). Adenoviruses are excreted in feces and go to the waste water and rivers where they live for a long time. The places of the first reproduction are the most often cells of epithelial conjunctiva, nasopharynx and intestine.

Adenovirus infection is clinically manifested by the fever, upper respiratory tract infections, tonsillitis, laryngitis, bronchitis and pneumonia. Conjunctivitis can be sometimes the dominant symptom, especially when the infection is acquired from the contaminated water during the bath. There can develop urethritis in the course of sexual intercourse. In infants and children, adenoviruses (types 40 and 41) usually cause heavy infections in the intestinal tract.

Adenoviral DNA diagnostics can be carried out from feces, urine, cerebrospinal fluid and swab from cornea, conjunctiva, urethra, cervix, rectum and nasopharynx.



Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Serum, plasma, CSF, swabs	Manual: EliGene [®] Urine Isolation Kit (ELISABETH PHARMACON) Automatic: ZEPHYRUS Magneto (ELISABETH PHARMACON)
Tissue, Biopsies	Manual: UltraClean Tissue DNA Kit Chemagic DNA Tissue10 Kit (PerkinElmer) Automatic: ZEPHYRUS Magneto (ELISABETH PHARMACON)
Feces	Manual: UltraClean Fecal DNA Kit

Serum, Plasma, CSF:

According to standard protocol take the sample of the serum, plasma or CSF into sterile tubes. Samples must be stored and transported at 4 °C. For the diagnostic purposes, it is recommended to isolate DNA from the sample during the day of taking sample. For DNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K to the sample and 20 µl of Internal Control DNA (IC DNA) and then continue according to the standard protocol of EliGene[®] Urine Isolation Kit for DNA isolation from blood. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C.

Automatic isolation:

Add 20 µl of Internal Control DNA (IC DNA) and isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Swabs:

These specimens should be collected according to standard protocol in collection tubes. Specimens should be transported to the laboratory at 4 °C. For the diagnostic purposes, it is necessary to make DNA isolation from the sample during the day of taking sample.

Manual isolation procedure is described in instruction manual of EliGene[®] Urine Isolation Kit.

1. Into 2.0 ml tube pipette 400 µl of MI3 solution, 20 µl of Proteinase K and 20 µl of Internal Control DNA (IC DNA).
2. Put the swab into the 2.0 ml tube and with sterile scissor cut the swab – cut about 0.5 cm above the swab. Close the tube.
3. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently shortly spin the tube.
4. By sterile pincers remove the swab and add 330 µl of solution MI4 to lysate. Vortex and shortly spin.
5. Continue according to the standard protocol of EliGene[®] Urine Isolation Kit. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C.

Automatic isolation:

1. Into 2.0 ml tube pipette 450 µl of Lysis buffer, 200 µl of PCR water, 10 µl of Proteinase K and 20 µl of Internal Control DNA (IC DNA).
2. Put the swab into the 2.0 ml tube and with sterile scissor cut the swab – cut about 0.5 cm above the swab. Close the tube.



3. Incubate tube 20 minutes at 65 °C in thermo shaker at 1000 rpm. Consequently shortly spin the tube.
4. Remove the swab by sterile pincers, vortex and shortly spin.
5. Pipette all volume of sample to position H at Deep well plate from MAGNETO BodyFluid DNA/RNA isolation kit.
6. Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Tissue, Biopsies:

Store the tissue immediately after the excise in the refrigerator at 4 °C and transport it to the laboratory during the same day.

Manual isolation:

For the diagnostic purposes, it is necessary to isolate DNA using UltraClean Tissue DNA Kit (MoBio) from the sample during the day of taking.

Add 20µl of Internal Control DNA (IC DNA) to the sample in the beating microtube. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C.

Automatic isolation:

Cut up to 10 mg tissue sample into small pieces, add 200 µl Lysis Buffer, 6 µl Proteinase K and 20 µl of Internal Control DNA (IC DNA) and isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to protocol with elution to 50 µl of Elution buffer.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems ABI 7000, 7300, 7500 (Applied Biosystems), LightCycler 480 and LightCycler Nano (Roche), RotorGene 6000 or RotorGene Q (Qiagen).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For ADV detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal Control the probe labeled with YakimaYellow is used (similar to VIC – exc. 525 nm – em. 548 nm, similar to JOE exc. 520 nm – em. 548 nm)
- Reaction Mix includes passive reference control dye ROX for signal normalization

RealTime Systems ABI 7000, 7300, 7500FAST (Applied Biosystems):

Use the program module for absolute quantification (Plate Type “Absolute Quantification” for ABI 7300, “Quantitation-Standard Curve” experiment for ABI 7500FAST). In case of ABI7500FAST use “7500 (96wells)” instrument type.



Set up the following temperature profile:

Holding stage

95°C 3 min

Cycling stage – 45 cycles

95°C 20 s

55°C 20 s

72°C 32 s Data collection ON

Collect emission signal at the third step – 72 °C.

The complete temperature profile can be up-loaded from Run Template “ADV_RT_ABI7300_v00.sdt” or “ADV_RT_ABI7500_v00.edt”. The Run Template can be copied from the CD included in the kit.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose “Dual Color Hydrolysis probe”.

Set up the following temperature profile:

Step 1 - Analysis mode “None”, 1 Cycle

95°C	3 min	Ramp rate (4.4°C/s)	Acquisition mode “None”
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Step 2 - Analysis mode “Quantification”, 45 Cycles

95°C	20 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
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55°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode “None”
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72°C	30 s	Ramp rate (4.4°C/s)	Acquisition mode “Single”
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Step 3 - Analysis mode “None”, 1 Cycle

40°C	1 min	Ramp rate (2.2°C/s)	Acquisition mode “None”
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The complete temperature profile can be up-loaded from Run Template “ADV_RT_LC480_v00.ix0”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

LightCycler® Nano (Roche):

For reaction use clear strips only.

In “Run Settings” menu choose “Hydrolysis Probes” option and “High Quality” option.

In “Profile menu” set up the following temperature profile:

Step 1 - Hold

95°C	3 min	Ramp rate (5°C/s)
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Step 2 – 3-Step Amplification, 45 cycles

95°C	20 s	Ramp rate (5°C/s)
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55°C	20 s	Ramp rate (4°C/s)	“Acquire” signal
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72°C	30 s	Ramp rate (5°C/s)
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Step 3 - Hold

40°C	1 min	Ramp rate (4°C/s)
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In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target 1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "ADV_RT_LCNANO_v00.ppf". The Run Template can be copied from the CD included in the kit.

RotorGene 6000 or Q (Qiagen):

In the "New Run" window choose "Two Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

95°C 3 min

Cycling stage – 45 cycles

95°C 20 s

55°C 20 s

72°C 30 s acquiring in channels "Green" and "Yellow"

The complete temperature profile can be up-loaded from Run Template "ADV_RT_RG_v00.ret". The Run Template can be copied from the CD included in the kit.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.
- Isolate DNA according to standard protocol.

Preparation of Reaction Mix

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

1. Detection: Take one microtube with Adenovirus RT Mix and after the thawing pipette 20 µl of Adenovirus RT Mix to amplification microtube or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of Adenovirus RT Mix, freeze it and store at -20 °C in a dark. Do not freeze tubes with Adenovirus RT Mix repeatedly. Under these conditions it is stable at least for 14 days.
2. Positive control: Take one microtube with Adenovirus RT Mix and after the thawing pipette 20 µl of Mix to amplification microtube or plate and add 5 µl of PC DNA Adenovirus. During the pipetting of positive control be careful to avoid contamination of other samples. Use separate pipette for positive controls!



3. Quantification Standards: Standards for the EliGene® Adenovirus RT kit must be ordered separately (EliDNA ADV QRT standard Cat. No. 90036-CL.QRT). Take one tube with Adenovirus RT Mix and after the thawing pipette 20 µl of mix to the amplification microtube or plate and add 5 µl of the standard of given concentration. Repeat this procedure with three other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used.

Insert the microtubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Result viewing

RealTime Systems ABI 7000, 7300, 7500FAST (Applied Biosystems):

In "Setup" software for RealTime PCR instrument set concentration of ADV Standards in FAM channel that are given on microtubes.

In "Analysis Settings" choose the "Auto Baseline" option for the calculation of the fluorescence back ground level (Baseline); - choose the "Manual Ct" option and set the "Threshold" for the YakimaYellow fluorescence to 0.03; choose the "Auto Ct" option for the FAM fluorescence.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in YakimaYellow channel (em. 548 nm).

The values of Qty correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in specific channel.

LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow. Enter the concentrations of ADV Standards in FAM channel that are mentioned on single microtubes

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

In Analysis window click to "Color Comp" icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon "Calculate".

Positive result: The positive results are characterized by amplification and growths of signal in FAM channel (465-510). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in YakimaYellow channel (533-580).

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.



LightCycler® Nano (Roche):

In "Samples" enter the concentrations of ADV Standards in FAM channel that are mentioned on single microtubes.

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (510-528). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in YakimaYellow channel (530-548).

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

RotorGene 6000 or Q (Qiagen)- version 1.7 and higher:

Click to "Edit Samples" icon in the menu and choose for Quantitation Standards Type "Standard" and for samples Type "Unknown". Enter the concentrations of ADV Standards in FAM channel that are mentioned on single microtubes. Click to "Analysis" icon in the menu and choose Analysis option "Quantitation". In "Quantitation Analysis" window choose "Dynamic Tube" and "Slope Correct" option.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (Green). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in YakimaYellow channel (Yellow).

The values of "Calc. conc." correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel does not appear before cycle number 45, the result of test should be interpreted as probably Adenovirus DNA negative or with concentration of Adenovirus DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive. This result does not exclude the occurrence of Adenovirus infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed Adenovirus DNA.

Positive result:

Amplification signal in FAM channel appears before cycle number 45. Adenovirus DNA was detected in the sample. The sample is Adenovirus DNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel (specific for Adenovirus) as well as increasing of amplification signal in YakimaYellow channel (specific for Internal Control) is not observed, it is necessary to repeat the analysis. The best would be to use new DNA prepared by new extraction process.



Control procedure

EliGene[®] Adenovirus RT Kit involves Internal Control DNA (IC DNA) and Positive Control (PC DNA Adenovirus). Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is Adenovirus DNA negative, the Cp of internal control must be $Cp < 35$. In the case of strongly positive samples usually the internal control amplification is not detected.

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 35 or less. The Cp higher than 35 for positive control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Usage the standards EliDNA ADV QRT standard:

Applied Biosystems RealTime System 7000, 7300, 7500 and 6000 or RotorGene RotorGene Q (Qiagen) instruments will perform the correlation coefficient calculation of standard curve labeled r by entering the appropriate values of calibrators. The value r of the correlation coefficient of standard curve must be higher than 0.99.

LightCycler[®] 480 instrument will perform the standard error calculation of standard curve labeled "Error" by entering the appropriate values of calibrators. The value "Error" of standard curve must be lower than 0.01.

In this case, the device managed on the basis of the measured results of calibrators build usable calibration line, whereby precisely subtract the results of other analyzed samples. Otherwise, it is necessary to repeat the analysis. Insufficient value of correlation coefficient or error can be caused by bad pipetting, insufficient vortexing of thawed calibrators or inappropriate storage of calibrators.

LightCycler[®] 480 instrument can perform evaluation by using external calibration curve. In this case, positive control at the concentration of 10 000 copies/ μ l as a standard can be used. However, we recommend a new calibration for each new shipment or with each new kit lot.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Reference material:

To monitor the all examination process covering DNA isolation and RealTime PCR detection is possible to use specimen positive for ADV DNA. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control DNA (IC DNA), there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.



Performance characteristics

Analytical performance characteristics:

Kit EliGene® Adenovirus RT has a very high sensitivity – detects 10 genomic DNA added to the amplification mix.

The sensitivity of test was verified as follows.

A specific-cloned insert of DNA (required concentration) was prepared and diluted to get desired concentrations of target sequence.

Analytical sensitivity is 10 copies of Adenovirus DNA in reaction mix.

Analytical specificity of method is 100%. Specificity of the methods was validated by searching the DNA databases and by addition of human DNA to MasterMix. 50 different samples of human DNA did not give false positive result. False positive result was not observed after the addition of DNA from these organisms *B. burgdorferi sensu lato*, *M. tuberculosis*, *M. bovis*, *M. cansasii*, *M. xenopii*, *M. avium*, *M. marinum*, *Lactobacillus*, *Enterococcus faecalis*, genus *Pseudomonas*, *E. coli*, *A. niger*, *C. albicans*, *S. aureus*, *S. agalactiae*, *N. gonorrhoeae*, *U. urealyticum*, *M. hominis* and *M. genitalium*, HBV, EBV, CMV, HSV1, HSV2, VZV.

Clinical specificity was tested on human blood and serum samples. 50 samples of different human DNA isolated from the blood and serum did not give false positive result.

Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® Adenovirus RT kit overall 96 clinical specimens of serum were analyzed. From these 96 clinical samples 83 samples were Adenovirus positive. Totally 13 specimens were right determined by EliGene® Adenovirus RT kit as Adenovirus negative.

The clinical sensitivity and specificity of EliGene® Adenovirus RT kit is 100%.

Measuring interval

The kit enables the detection of 10^1 – 10^8 of viral DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal Control DNA (IC DNA) for checking the process of DNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

Unused content of the tube with MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.



Warnings and general precautions

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.



- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mix (Adenovirus RT Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA are disposable and therefore must be used once only in the preparation of the reaction mixture.
- These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Literature

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

Jothikumar N, Cromeans TL, Hill VR, Lu X, Sobsey MD, Erdman DD. 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Appl Environ Microbiol. 71(6):3131-6



Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

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