DNA diagnostics EliGene®

CE

EliGene® Adenovirus RT

REF **90036-RT (for 50 samples)**

Intended use:
EliGene® Adevirus RT kit is qualitative in vitro diagnostic device intended for the detection of adenovirus in clinical material.
EliGene® Adenovirus RT kit has a very high sensitivity that allows to detect even 10 genomic DNA in the mastermix.
Specificity of the kit: kit provides positive result only for adenoviruses respectively for all 51 types of adenoviruses.

Introduction:
Adenoviruses represent the largest nonenveloped 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 up of 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 place of the first reproduction is the most often cells of epithelial conjunctiva, nasopharinx 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, cerebrospinal fluid and swab from cornea, conjunctiva, urethra, cervix, rectum and nasopharynx.

Kit components:
5 x 200 µl **ADV Mix**
1 x 125 µl Positive Control DNA – **PC DNA ADV**
5 x 200 µl Internal Control DNA – **IC DNA ADV**
Instruction for use

Specimen:
For diagnostic purposes following clinical specimen are recommended a) cerebrospinal fluid b) feces c) swab from cornea, conjunctiva, urethra, cervix, rectum and nasopharynx .

Clinical material:	Recommended DNA isolation procedure:
CSF	UltraClean BloodSpin Kit (MoBio) chemagic Viral DNA/RNA Kit (chemagen)
Feces	UltraClean Fecal DNA Kit (MoBio)
Swabs	UltraClean DNA Tissue Kit (MoBio) + EliDNA Store Kit (ELISABETH PHARMACON) chemagic DNA Tissue40 Kit (chemagen)

CSF: Take the CSF according to standard procedure. The sample is necessary to be transported and to be stored at 4°C or frozen at -20°C. The kit is optimized for use of DNA isolation kit UltraClean BloodSpin Kit (MoBio) or alternatively chemagic Viral DNA/RNA (chemagen). **Before the isolation 20 µl of Inhibition Control DNA (IC DNA ADV) must be added to 200 µl of CSF sample.** Use isolated DNA for the detection immediately after the isolation or store DNA hours to one day at 4°C or freeze DNA at -20°C for longer time. The concentration of isolated DNA is getting lower in every freezing process and there is a risk of false negative results. That is the reason why the PCR should be carried out immediately after the DNA isolation.

Feces: Isolate viral DNA from the stool according to standard procedure using UltraClean Fecal DNA Kit (MoBio). After the homogenization of adequate amount of the sample, it is necessary to add **20 µl of Inhibition Control DNA (IC DNA ADV).**

Swabs: Obtain the swab according to standard procedure. The sample is necessary to be transported and to be stored at 4°C or frozen at -20°C. The kit is ideally optimized for use of EliDNA store kit which preserves the taking sample at the room temperature for a few days. The kit is also optimized for use of DNA isolation kit UltraClean DNA Tissue Kit (MoBio) or alternatively chemagic DNA Tissue40 Kit (chemagen). After the homogenization of adequate amount of the sample is necessary to add **20 µl of Inhibition Control DNA (IC DNA ADV).** Use isolated DNA for the detection immediately after the isolation or store DNA hours to one day at 4°C or freeze DNA at -20°C for longer time. The concentration of isolated DNA is getting lower in every freezing process and there is a risk of false negative results. That is the reason why the PCR should be carried out immediately after the DNA isolation.

Essential equipment in laboratory:
Sterile 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 Real-Time PCR – the kit is designed for Real-Time Systems 7000, 7300, 7500 from Applied Biosystems and is fully compatible with RotorGene instruments without other processing.
Sterile microtubes (DNase-, RNase-free) compatible with given Real-Time system.

Storage and expiration date:
All components of the kit must be transported and stored at -20° C. Once the reagents are thawed do not freeze them again and store them at 4°C. Under these conditions they are stable at least for 14 days. If after the analysis remaining part of the reagents aliquots do not freeze them and store them in fridge until the next analysis. Under these conditions they are fully functional at least for 14 days. Kit must be store in a dark.

Configuration of Real Time instrument:
Use the program module for absolute quantification (Plate Type "Absolute Quantification")

1x	50° C 2 min	1x	95° C 10 min	45x	95° C 20 s 55° C 20 s 72°C 32 s

For ADV detection probe labeled with FAM is used (abs. 494nm – emission 518nm).
For inhibition control (IC) probe labeled with Yakima Yellow is used (similar to VIC – abs. 525nm – emission 548 nm, similar to JOE abs. 520nm – emission 548nm).
Collect emission signal scan at the third step 72° C.
Mastermixes include passive reference control dye ROX for signal normalization.
Mastermixes include amplificate contamination protection.

Procedure:

1. During the isolation, 20 µl of Internal Control DNA (IC DNA ADV) must be added to the sample. Carry out the isolation of DNA by optimized protocol mentioned above.
2. Own detection: Take one microtube with ADV mix and after the thawing, pipette 20 µl of mix to amplification tube and add 5 µl of isolated DNA. Tube with ADV mix does not freeze again and if you do not use the content, store it at 4° C in a dark.
3. Positive control: Take off one microtube with ADV mix, pipette 20 µl of mix to amplification tube and add 5 µl of positive control from Positive control DNA (PC DNA ADV) tube.
4. Insert the micro tubes to the Real-Time PCR instrument and run the program.

Results viewing:
Positive result is characterized by increasing of amplification signal in FAM channel. When the result is negative or slightly positive the amplification of internal control must be observed in Yakima Yellow (eventually VIC) channel.
For the RealTime System 7000, 7300 and 7500 use the automatic analysis. The values of Ct correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in specific channel.

Results interpretation:
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 adenoviral DNA negative. 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 adenoviral DNA.

Positive result
Adenoviral DNA was detected in the sample. The sample is adenovirus DNA positive.

Alert: 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 of specific signal for adenovirus in FAM channel and also increasing of amplification signal for internal control (in Yakima Yellow channel) is not observed, it is necessary to repeat the analysis. It is the best to use DNA samples prepared by new DNA isolation.

Quality control:
EliGene® Adenovirus RT Kit involves internal isolation control and positive control. 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 ADV DNA negative, the Ct of internal control must be Ct < 30.**
Positive control follows the proper function of mastermix. **Minimal Ct of positive control must be 20 or lesser. The Ct higher than 20 for positive control can't be accepted and DNA detection must be repeated with new sample.** In the case of repeatedly lower Ct 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.

Troubleshooting:
1. If there is no amplification of inhibition control, there is some problem in isolation of DNA or the kit is after the expiration date or there is Real-Time instrument breakdown.
2. If there is no amplification of positive control, the kit is after the expiration date or there is Real-Time instrument breakdown.

Principle of the method:
This diagnostic kit uses primers and TaqMan probes (labeled by FAM and YakimaYellow).

Functional 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. 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. Moreover, addition of DNA from *M. tuberculosis, M. cansasii, M. xenopii, M. avium* a *M. marinum,* HBV, EBV, CMV, VZV, HSV2, *C. trachomatis, E. coli, A. niger, C. albicans* did not give false positive result too.

Repeatability of the method was tested with 10 positives and 10 negatives samples for three consecutive days. The same lot of mastermix was used. The results were same in all performed tests.

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

Warning:
After thawing Mastermixes are stable for 2 weeks at 4°C. Do not freeze Mastermix again! Do not mix components of the kits from different lots.

Limitations of the method:

This product must be handled by personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid result errors. It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results or false positive results caused by wrong taking, transporting or wrong sample processing. As with any diagnostic device, the results obtained with this product must be interpreted in consideration of all the clinical data and other laboratory tests done on the patient. As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product. This residual risk cannot be eliminated or reduced any further. In particular situations such as emergency diagnoses, this residual risk can contribute to incorrect decisions with potentially grave consequences for the patient.

Warnings and general precautions

This kit is intended for *in vitro* use only.

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 put into autoclave and 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 manufacturers' kits.

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 flow hood. Tubes containing different samples must never be 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 a laminar flow hood. 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 a 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 mixes (ADV Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.

These mixes carry the following safety warnings (S):
S 23-25. Do not breathe gas/fumes/vapor/spray. Avoid contact with eyes.

The tubes containing mixes (Inhibition Control DNA – IC DNA ADV, Positive Control DNA – PC DNA ADV) are disposable and therefore must be used once only in the preparation of the reaction mixture. These mixes carry the following safety warnings (S):

S 23-25. Do not breathe gas/fumes/vapour/spray. Avoid contact with eyes.

In the case of any problems call our customer support.

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

Producer:
ELISABETH PHARMACON, spol. s r.o.

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