Adenovirus IgA Human ELISA Kit

Cat. No.:DEIA1383
Pkg.Size:96T

Intended use

For the qualitative determination of IgA class antibodies against Adenovirus in Human serum or plasma (citrate).

General Description

Adenoviruses are double-stranded DNA viruses of about 70-90 nm lacking an envelope. The capsid contains 252 capsomeres and shows icosahedral symmetry. The capsomeres consist of hexons, pentons and fiberprotein trimers which are responsible for the induction of group- and type-specific antibodies. For the first time adenoviruses were isolated in 1953 from tonsils and adenoid tissue by Rowe. More than 80 adenoviruses are known at present. 47 out of them are pathogenic for men. They cause several diseases of different organ systems, mainly eyes, pharynx, respiratory and gastrointestinal system.

Reagents And Materials Provided

Adenovirus Coated Wells (IgA): 12 break apart 8-well snap-off strips coated with Adenovirus antigen; in resealable aluminium foil.
• IgA Sample Diluent ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
• Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
• Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
• Adenovirus anti-IgA Conjugate**: 1 bottle containing 20 ml of peroxidase labeled rabbit antibody to human IgA; colored violet, ready to use; black cap.
• TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'- tetramethylbenzidine (TMB); ready to use; yellow cap.
• Adenovirus IgA Positive Control***: 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
• Adenovirus IgA Cut-off Control***: 1 bottle containing 3 ml; colored yellow; ready to use; green cap.
• Adenovirus IgA Negative Control***: 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.
• Strip holder: 1
• Cover foil: 1
* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

Materials Required But Not Supplied

• ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
• Incubator 37°C
• Manual or automatic equipment for rinsing wells
• Pipettes to deliver volumes between 10 and 1000 µl
• Vortex tube mixer
• Deionised or (freshly) distilled water

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• Disposable tubes
• Timer

Storage

The reagents are stable up to the expiry date stated on the label when stored at 2-8°C.

Specimen Collection And Handling

Sample Dilution:
Before assaying, all samples should be diluted 1+100 with IgA Sample Diluent. Dispense 10µl sample and 1ml IgA Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Reagent Preparation

It is very important to bring all reagents, samples and standards to room temperature (20-25°C) before starting the test run.
1. Coated snap-off Strips: The ready to use break apart snap-off strips are coated with Adenovirus antigen. Store at 2 - 8 °C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8 °C; stability until expiry date.
2. Adenovirus anti-IgA Conjugate: The bottle contains 20 ml of a solution with anti-human-IgA horseradish peroxidase, buffer, stabilizers, preservatives and an inert violet dye. The solution is ready to use. Store at 2 - 8°C. After first opening stability until expiry date when stored at 2 - 8 °C.
3. Controls: The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2 - 8°C. After first opening stability until expiry date when stored at 2 - 8°C.
4. IgA Sample Diluent: The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the specimen. This ready to use solution has to be stored at 2 - 8°C. After first opening stability until expiry date when stored at 2 - 8°C.
5. Washing Solution (20x conc.): The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. After first opening stability until expiry date when stored at 2 - 8°C.
6. TMB Substrate Solution: The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2 - 8°C away from the light. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2 - 8°C.
7. Stop Solution: The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2 - 8°C. After first opening stability until expiry date.

Assay Steps

Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300 µl to 350 µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.
Please allocate at least:
1 well (e.g. A1) for the blank
1 well (e.g. B1) for the negative control

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2 wells (e.g. C1+D1) for the cut-off control and
1 well (e.g. E1) for the positive control
• It is recommended to determine controls and samples in duplicate, if necessary.
• Perform all assay steps in the order given and without any appreciable delays between the steps.
• A clean, disposable tip should be used for dispensing each standard and each sample.
• Adjust the incubator to 37° ± 1°C.
Assay Procedure:
1. Dispense 100 µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour ± 5 min at 37±1°C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100 µl Adenovirus anti-IgA Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. Incubate for 30 min at room temperature. Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. Incubate for exactly 15 min at room temperature in the dark.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.
Note: Highly positive samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.
11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

Calculation

The cut-off is the mean absorbance value of the Cut-off control determinations.
Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 =0.76 / 2 = 0.38
Cut-off = 0.38

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is > 90%.

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is > 90 %.

Reproducibility

Reproducibility

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Inter-assay	n	Mean	Cv (%)
Pos. Serum	12	2.85	3.8

Intra-assay	n	Mean	Cv (%)
Pos. Serum	20	1.27	2.8

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

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