Adenovirus Hexon Antigen Capture ELISA

Product Insert

Cat. No. AK290-2 (2 x 96 wells)

For Research Use Only

Introduction

The Virusys Adenovirus Hexon Antigen Capture ELISA is a qualitative procedure for the determination of adenovirus hexon antigen in a variety of sample matrices, including feces. The assay is a double antibody (sandwich) ELISA that utilizes a monoclonal anti-adenovirus antibody to capture the antigen from the sample. Following sample incubation, a biotinylated detection antibody is added and this step is followed by HRP-Streptavidin. The presence of hexon antigen is visualized by the addition of an HRP substrate followed by the addition of a stop reagent. The development of color within the well is indicative of the presence of adenovirus hexon antigen in the sample. For quantitative purposes, Virusys offers a separate calibration kit (AK291, Adenovirus Antigen Calibration Kit) which can be used in conjunction with this product to generate a standard curve for quantitative measurement.

Acute diarrheal disease in young children is a major cause of morbidity worldwide and is a leading cause of mortality in developing countries [8]. Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhea in many of these children, second only to the rotaviruses. [1,3,5-8] These viral pathogens have been isolated throughout the world, and can cause diarrhea in children year round.[1-4] Infections are most frequently seen in children under two years of age,[1-3] but have been found in patients of all ages.[2] Further studies indicate that adenoviruses are associated with 4 - 15% of all hospitalized cases of viral gastroenteritis.[1-8]

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Many laboratories use electron microscopy (EM) to detect viruses associated with gastroenteritis [5, 7, 8]. Other techniques include direct genome profiling and nucleic acid hybridization, neither of which is rapid or specific [6]. Alternatively, ELISA tests using Adenovirus-specific antibodies have been shown to be a sensitive [9], specific, and rapid methods for the detection of enteric adenoviruses[6].

The test kit incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from complex sample matrix effects and/or the nonspecific adsorption of reactive test components which result in improvements in both sensitivity and specificity. The kit is available in a standard photometric detection format (AK290-2 or AK290-5).

The kit has been tested against a variety of adenovirus subtypes for sensitivity and potentially interfering viruses and bacteria for specificity.

Components

1. Adenovirus Antigen Capture Plate (96 tests)-2 ea.
2. Sample Preparation Reagent (1x)-12 ml
3. Adenovirus Positive Control (1x)-1 ml
4. Adenovirus Negative Control (1x)- 2 x 1.5 ml
5. Wash Buffer (20x)-2 x 50 ml
6. Adenovirus Detection Antibody, Biotin-labeled (1x)-22 ml
7. Streptavidin-HRP (1x)-22 ml
8. Chromagen Solution (1x)-22 ml
9. Stop Solution (1x)-22 ml
10. Sample Dilution Tray-2 ea.

Optional Components

AK291--Adenovirus Antigen Calibration Kit

Storage

Store all kit components at 2-8° C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

Procedure

1. Remove the kit from storage and allow it to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample. In addition, include one well for the **Adenovirus Positive Control** and three wells for the **Adenovirus Negative Control**.

3. To begin the assay, transfer 50 µl of **Sample Preparation Reagent** to the appropriate number of wells in the dilution tray provided.
4. Add 200 µl of each sample, positive control, and negative control to the Sample Preparation Reagent. Mix by pipetting up and down several times.
5. Transfer 100 µl of sample or control to the appropriate wells of the **Adenovirus Antigen Capture Plate**.
6. Cover the plate and incubate for 30 min. at room temperature on a plate shaker set at moderate speed.
7. Empty the wells and wash 5x with at least 300 µl/well **1x Wash Buffer**.
8. Add 100 µl of **Adenovirus Detection Antibody** to each well. Cover the plate and incubate for an additional 30 min. on a plate shaker using the same settings (Step 6).
9. Wash the wells 5x with at least 300 µl/well **1x Wash Buffer**.
10. Add 100 µl of **Streptavidin-HRP** to each well.
11. Cover the plate and incubate for 30 min. on a plate shaker using the same settings as in Step 6.
12. Wash the wells 5x with at least 300 µl/well **1x Wash Buffer**.
13. Add 100 µl of **Chromagen** to each well and incubate for 10 min. on a plate shaker.
14. Stop the reaction by the addition of 100 µl of **Stop Solution**.
15. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

Quality Control

1. All negative control absorbance values should be ≤0.250.
2. The positive control absorbance value should be ≥0.500.
3. The calculated value for the positive control/cut-off should be ≥2 (see below).

Determination of Cut-off and Interpretation of Results

1. To determine the **cut-off value**, calculate the mean of the three negative control absorbance values and multiply this value by 2.
2. To interpret the results for a given sample, divide the absorbance value for the sample by the cut-off value. Calculated sample values that are >1.1 are considered reactive. Calculated sample values that are <0.9 are considered nonreactive. Calculated sample values that are ≥0.9 and ≤1.1 are considered equivocal.

References

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