Adenovirus Hexon ELISA Kit

Adenovirus Hexon ELISA Kit is an Enzyme Immunoassay kit for the quantification of Adenovirus Hexon in cell culture supernatants.

Catalog number: ARG83386

Package: 96 wells

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INTRODUCTION

In molecular biology, the hexon protein is a major coat protein found in adenoviruses. Hexon coat proteins are synthesised during late infection and form homo-trimers. The 240 copies of the hexon trimer that are produced are organised so that 12 lie on each of the 20 facets. The central 9 hexons in a facet are cemented together by 12 copies of polypeptide IX. The penton complex, formed by the peripentonal hexons and penton base (holding in place a fibre), lie at each of the 12 vertices. The hexon coat protein is a duplication consisting of two domains with a similar fold packed together like the nucleoplasmin subunits. Within a hexon trimer, the domains are arranged around a pseudo 6-fold axis. The domains have a beta-sandwich structure consisting of 8 strands in two sheets with a jelly-roll topology; each domain is heavily decorated with many insertions. Some hexon proteins contain a distinct C-terminal domain.

Hexon directly recruits the cellular motor protein dynein in a pH-dependent manner. The dynein-regulatory protein, dynactin, was found to play a clear role in regulating the dynein-adenovirus complex transport to the nucleus.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Adenovirus Hexon has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Adenovirus Hexon present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Adenovirus Hexon is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish

Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Adenovirus Hexon bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Adenovirus Hexon in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard at ≤ -20°C

Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
Standard	100 µL (1 µg/mL)	-20°C
1000X Adenovirus Hexon Antibody	20 µL	4°C
1000X HRP-Streptavidin Solution	20 µL	4°C
Diluent Buffer	50 mL	4°C
10X Viral Lysis Buffer	15 mL	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard at ≤ -20°C, Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.

- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Sample - Transfer 225 µL of and 25 µL of 10X Lysis Buffer for each sample to a microcentrifuge tube, vortex well. Inactivate Adenovirus sample at 56°C for 30 min. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as adenoviral lysate.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **10X** Wash Buffer into distilled water to yield 1X Wash Buffer.
- **1X Adenovirus Hexon Antibody:** 10 minutes before use, dilute **1000X** Adenovirus Hexon Antibody into Diluent Buffer to yield **1X** Adenovirus Hexon Antibody.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield **1X** HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Standard:** Spin down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	Adenovirus Hexon (ng/mL)	Diluent Buffer (µL)	Standard (µL)
S1	10	990	10 (1 µg/mL)
S2	5	500	500 of S1
S3	2.5	500	500 of S2
S4	1.25	500	500 of S3
S5	0.625	500	500 of S4
S6	0.3125	500	500 of S5
S7	0.1562	500	500 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

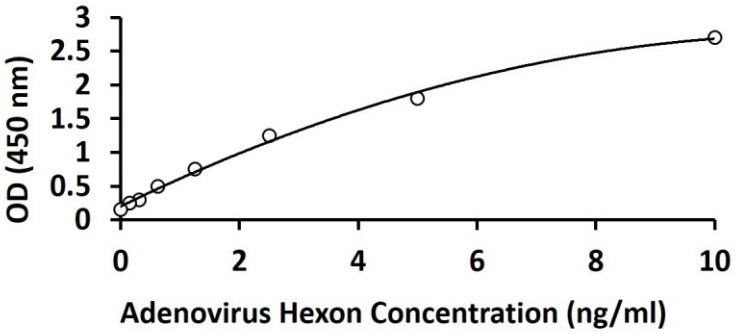
1. Add **100 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **2 hour** at **37°C.**
3. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Adenovirus Hexon Antibody** to each well.
5. Cover the plate and incubate for **1 hour** at **RT**.
6. Aspirate each well and **wash** plate **as step 3**.
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
8. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
9. Aspirate each well and **wash** plate **as step 3**.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **2-30 mins** at **room temperature** in the dark.
12. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Adenovirus Hexon ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.1 ng/mL

Assay Range

0.156- 10 ng/mL