Creative Diagnostics®

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User's Manual

GTCDx™ Human Anti-AAV1 Antibody ELISA Kit

REF **DEIASL342**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Anti-AAV1 Antibody ELISA is used as an analytical tool for qualitative determination of antibodies to AAV1 in serum and plasma.

General Description

Researchers have used AAV-based vectors in pre-clinical research and in clinical trials in which AAV-based vectors have demonstrated a good safety profile. AAVs have also demonstrated lasting therapeutic gene expression following a single treatment in preclinical and clinical studies.

Scientists today have used the AAV1 variant, or serotype, of vector in potential product candidates in hemophilia B and Huntington's disease. Research suggests that a greater portion of patients can be effectively treated with AAV1 gene therapy, compared to other AAV-based gene therapies.

AAV1 gene therapies present a favorable immunogenicity profile. To date, insect-cell manufactured AAV1 gene therapies have been administered to 22 patients across three clinical studies by a leading gene therapy company without any evidence of AAV1 capsid-specific cellular immune responses or long-term safety complications.

One of the major challenges in AAV-based gene therapy is the presence of circulating anti-AAV neutralizing antibodies, which can pre-exist in patients and may prevent successful gene transfer. High levels of circulating anti-AAV neutralizing antibodies can develop after a single administration of gene therapy and can prevent successful gene transfer in patients.

Principles of Testing

The method employs the qualititative sandwich enzyme immunoassay technique. AAV1 capsid protein is precoated onto microwells. Samples and control are pipetted into microwells and Antibodies to AAV1 present in the sample are bound by the capture protein. After incubation, washing is done to remove the unbound Anti-AAV1. AAV1-HRP conjugate is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of antibodies to AAV1 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Reagents And Materials Provided

1. AAV1 Capsid Coated Microtiter Plate (12x8 wells) – 1 no
2. Negative Control – 1 ml
3. Anti-AAV1 Positive Control – 1 ml
4. AAV1-HRP Conjugate – 12 ml
5. Assay Diluent – 6 ml
6. Sample Diluent – 50 ml
7. Wash Buffer (20X) – 50 ml

8. TMB Substrate – 12 ml
9. Stop Solution – 12 ml
10. Instruction Manual

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Storage

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation- Samples have to be diluted 1 in 400 (v/v), e.g. 5 ul sample in 2 ml sample diluent, prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Plate Preparation

A typical Assay Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	N.C.	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43
B	N.C.	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
C	P.C.	S4	S8	S12	S16	S20	S24	S8	S32	S36	S40	S44
D	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
E	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
F	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	N.C.
G	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	P.C.
H	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	P.C.

* All controls and samples are run in duplicates

Blank – Blank wells

P.C – Positive control wells

N.C – Negative control wells

S. – Sample extract wells

Reagent Preparation

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 50 ml of 20X Wash Buffer in 950 ml of DI water.

Assay Procedure

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Anti-AAV1. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-AAV1 present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Anti-AAV1 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing Sodium Azide (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-AAV1.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Steps:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37ºC.
2. Pipette out 50 ul of Assay Diluent in each well.
3. Add 100 ul of Negative Control, Anti-AAV1 Positive Control and diluted Samples into respective wells.
4. Cover the plate and incubate for 60 minutes at 37°C.
5. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Pipette without delay in the same order 100 ul of AAV1-HRP Conjugate into each well.
7. Cover the plate and incubate for 30 minutes at 37°C.
8. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
9. Add 100 ul of TMB Substrate in each well.
10. Incubate the plate for 30 minutes at 37°C in dark. Positive wells should turn bluish in color. (DO NOT SHAKE or else it may result in higher backgrounds and worse precision).
11. Pipette out 100 ul of Stop Solution. Wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate reader.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Interpretation Of Results

Results are interpreted qualitatively by calculating a cut-off value. The same maybe done as under –

Read the Sample and Negative Control wells on microtiter plate reader at 450nm. The OD (Optical Density) of NC (Negative Control) in triplicate should be used for calculating the mean and standard deviation. This is the $Blank_{mean}$. The cut-off for Positives is equal to a value greater than ($Blank_{mean}$ + 2*Standard Deviation).

Formula:

Positive Sample Value = OD > ($Blank_{mean}$ + 1 or 2 * SD)

1= If samples are in single wells

2= If samples are in duplicates

3= If samples are in triplicates

For Example –

Sample Type	Absorbance #1	Absorbance #2	Absorbance #3	Mean
Negative	0.200	0.219	0.221	0.213
Standard Deviation	0.200-0.213 = -0.013	0.219-0.213 = 0.006	0.221-0.213 = 0.008	

Mean Standard Deviation = $\sqrt{(-0.013)^2 + (0.006)^2 + (0.008)^2 / n-1} = 0.0082$

Therefore Cut-Off = Mean + 2 * SD
= 0.213 + 2 * 0.0082
= 0.213 + 0.0164
= 0.229 say 0.23

Typical Reference Values would then be:

Note: The standard deviation (SD) provides an estimate of the reproducibility of replicate data points and can provide confidence levels for assessing if one value is truly different from another. Whatever the measured value, a certain percentage of the values obtained are contained within the standard deviation.

For instance, one SD on either side of the mean contains 68% of the values under the curve of that distribution. Approximately two SD (actually 1.96 SD) on either side of the mean contains 95% of all of the values and approximately three SD (actually 2.58 SD) contains 99% of all values. Thus if a value that is greater than three SD different from the mean of a set of samples is obtained, one can be 99% confident that it is truly different from the first set of samples. Mathematically, the SD is the square root of the sum of the variances squared divided by the number of samples minus one.

Precautions

1. This Kit is For Research Use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reconstituted reagents.
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept at 2 - 8 °C before use in the original shipping container.
7. Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed
   - Do not smoke, eat or drink while handling kit material
   - Always use protective gloves - Never pipette material by mouth
   - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
10. In any case GLP should be applied with all general and individual regulations to the use of this kit.