



User's Manual

GTCDx™ Canine Anti-AAV5 Antibody ELISA Kit



DEIASL343D



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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.

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PRODUCT INFORMATION

Intended Use

The Canine anti-AAV5 ELISA is used as an analytical tool for qualitative determination of antibodies to AAV5 in Canine serum.

Principles of Testing

The qualitative immune enzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

AAV5 capsid protein is precoated onto microwells. Samples and control are pipetted into microwells and antibodies to AAV5 present in the sample are bound by the capture protein. After incubation, washing is done to remove the unbound Anti-AAV5. An enzyme linked polyclonal antibody specific for canine IgG 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 AAV5 in the sample. Color development is then stopped by addition of stop solution. Absorbance at 450/620 nm is read using an ELISA Microtiter plate reader.

Reagents And Materials Provided

1. AAV5 Capsid Coated Microtiter Plate, 12x8 wells
2. Negative Control, ready to use, 1 × 1 ml
3. Canine Anti-AAV5 Positive Control, ready to use, 1 × 1 ml
4. Anti-canine IgG Conjugate, ready to use, 1 × 12 ml
5. Sample Diluent, ready to use, 1 × 50 ml
6. Wash Buffer, 20 × concentrate, 1 × 50 ml
7. TMB Substrate, ready to use, 2 × 6 ml
8. Stop Solution, ready to use, 1 × 7 ml
9. Instruction Manual

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm, with the correction wavelength set at 620 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. Timer
6. Absorbent Paper

Storage

1. All reagents should be stored at 2°C to 8°C 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 (18-25°C). 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

1. Serum

Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 10-15 minutes at 1500 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. **Note: Lipaemic, hemolytic or contaminated samples should not be run.**

Sample Preparation Before Use

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

Samples have to be diluted 1 in 50 (v/v), e.g. 10 µl sample in 490 µl 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

	1	2	3	4	5	6	7	8	9	10	11	12
A	N.C.		S7		S15		S23		S31		S39	
B	P.C.		S8		S16		S24		S32		S40	
C	S1		S9		S17		S25		S33		S41	
D	S2		S10		S18		S26		S34		S42	
E	S3		S11		S19		S27		S35		S43	
F	S4		S12		S20		S28		S36		S44	
G	S5		S13		S21		S29		S37		S45	
H	S6		S14		S22		S30		S38		S46	

* All controls and samples are run in duplicates

P.C. – Positive control

N.C. – Negative control

S. – Sample

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 (1×): dilute 50 ml of 20× 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-AAV5. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-AAV5 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 AAV5 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 (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-AAV5.
4. It is recommended that all Controls 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 Controls and Samples.

Assay Steps:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at 37°C.
2. Add 100 µl of Canine Anti-AAV5 Positive Control, Negative Control, and diluted Samples into respective wells.
3. Cover the plate and incubate for 30 minutes at 37°C.
4. 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.
5. Pipette without delay in the same order 100 ul of Anti-canine IgG Conjugate into each well.
6. Cover the plate and incubate for 30 minutes at 37°C.
7. 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.
8. Add 100 ul of TMB Substrate in each well.
9. Incubate the plate for 15 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).
10. Pipette out 50 ul of Stop Solution. Wells should turn from blue to yellow in color.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

Calculation

Determine the Mean Absorbance for each set of duplicate control and Samples.

Calculate average (Avg) OD: Avg OD = Average OD from AAV5 Capsid Coated Microtiter Plate

Avg OD of the Canine Anti-AAV5 Positive Control should be ≥ 0.3 .

Avg OD of the Negative Control should be < 0.1 .

CUT OFF value = Negative Control + 0.1.

Interpretation Of Results

If the Positive Control value is ≥ 0.3 , and the Negative Control is < 0.1 , the test is valid, otherwise, the test is invalid.

If Avg OD of Samples $<$ CUT OFF, the test samples are considered negative.

If Avg OD of Samples \geq CUT OFF, the test samples are considered positive.

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. 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.
8. 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.
9. In any case GLP should be applied with all general and individual regulations to the use of this kit.