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User's Manual

Mouse Anti-AAV8 ELISA Kit(Quantitative)

REF **DEIASL345MQ**

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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.

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PRODUCT INFORMATION

Intended Use

The Mouse Anti-AAV8 ELISA is used as an analytical tool for quantitative detection of antibodies to AAV8 in Mouse serum.

Principles of Testing

This ELISA is designed, developed and produced for quantitative measurement of mouse anti-AAV8 IgG in test sample. The assay utilizes the indirect enzyme linked immunosorbent technique.

AAV8 capsid protein is precoated onto microwells. Samples and control are pipetted into microwells and antibodies to AAV8 present in the sample are bound by the capture protein. After incubation, washing is done to remove the unbound Anti-AAV8. An enzyme linked polyclonal antibody specific for mouse 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 AAV8 in the sample. Color development is then stopped by addition of stop solution. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

Reagents And Materials Provided

1. AAV8 Capsid Coated Microtiter Plate, 12x8 wells
2. Mouse Anti-AAV8 Standard (2.5 µg/mL), 120µL
3. Anti-Mouse IgG Conjugate, 100 × concentrate, 120µL
4. Sample Diluent, ready to use, 1 × 50 mL
5. Wash Buffer, 20 × concentrate, 1 × 50 mL
6. TMB Substrate, ready to use, 2 × 6 mL
7. Stop Solution, ready to use, 1 × 7 mL
8. 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 µl to 1000 µl
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 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

Collection and storage

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 x 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

Test Sample preparation- 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 20 (v/v), e.g. 10 µl sample in 190 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.

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.
4. To make Anti-mouse IgG Conjugate (1×), dilute 10 µl of 100× Anti-mouse IgG Conjugate in 990 µl of Sample Diluent.
5. Standard Preparation: Please prepare 6 tubes labeled as S0-S5 and use the Stock (Mouse Anti-AAV8 Standard (2.5 µg/mL)) to produce a quadruple dilution series according to the picture shown below.

Suggested Preparation of Standards			
	ng/ml	Range: 0.98 to 250 ng/ml	
Stock	2500		
S1	250	Add 50µl Stock	+450µl Sample Diluent
S2	62.5	Add 100µl S1	+300µl Sample Diluent
S3	15.6	Add 100µl S2	+300µl Sample Diluent
S4	3.91	Add 100µl S3	+300µl Sample Diluent
S5	0.98	Add 100µl S4	+300µl Sample Diluent
S0	0		300µl Sample Diluent

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-AAV8. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-AAV8 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-AAV8 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-AAV8.
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 standards and samples be run in duplicates or triplicates. All steps must be performed at 37°C.
2. Add 100 µl of standards and diluted samples into respective wells.
3. Cover the plate and incubate for 30 minutes at 37°C.
4. Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 350 µL Wash Buffer (1×) at interval of 30s for 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
5. Pipette without delay in the same order 100 µl of Anti-mouse IgG Conjugate (1×) into each well.
6. Cover the plate and incubate for 30 minutes at 37°C.
7. Repeat the wash step as described in step (4).
8. Add 100 µl of TMB Substrate in each well.
9. Incubate the plate for 15 minutes at 37°C in dark. (DO NOT SHAKE or else it may result in higher backgrounds and worse precision).
10. Terminate reactions by adding 50 µl Stop Solution per well. 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.

Quality Control

Determine the Mean Absorbance for each set of duplicate standards and samples.

Calculate average (Avg) OD: Avg OD = Average OD from AAV8 Capsid Coated Microtiter Plate

Avg OD of the 250 ng/ml standard should be ≥ 2.0

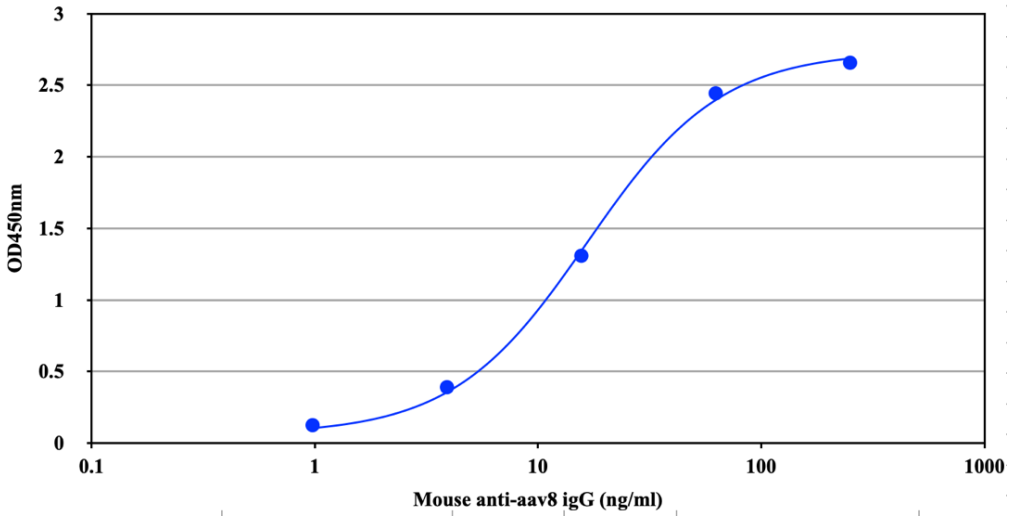
Avg OD of the 0 ng/ml standard should be < 0.1.

If the 250 ng/ml standard value is ≥ 2.0, and the 0 ng/ml standard is < 0.1, the test is valid, otherwise, the test is invalid.

Calculation

1. For the calculation of the sample concentration, polynomial regression is recommended. Standard curve is constructed by plotting the units of the 6 standard points (ng/mL) along the abscissa (X axis) and the corresponding OD450nm values along the ordinate (Y axis).
2. The mouse anti-AAV8 IgG concentration of samples can be reported based on the principles indicated below.
3. The mouse anti-AAV8 IgG concentration of pre-diluted (at the ratio of 1:20) samples can be directly read on the standard curve. In order to report concentration for the corresponding undiluted sample, the value must be multiplied by the factor of 20. Example: If a 1:20 pre-diluted sample results in a concentration of 100 ng/mL on the standard curve, then its undiluted sample value corresponds to 2000 ng/mL (100 ng/mL x 20 = 2000 ng/mL).
4. If any diluted sample is reading OD450nm higher than that of highest standard supplied, it should be further diluted (in such a case 1:100 dilution of the sample is proposed) appropriately with Sample Diluent and then retested. Also, this second dilution has to be used for calculation of the final result. It is recommended that each laboratory determines the best dilution ratio for their samples in order to minimize retesting.

Typical Standard Curve



Precision

Intra-Assay: CV < 15%

Inter-Assay: CV < 15%

Detection Range

For mouse anti-AAV8 IgG in serum, the method has been demonstrated to be highly (>0.99) linear from 0 to 250 ng/mL.

Sensitivity

The Detection Threshold for the assay is 1.72 ng/mL. The assay sensitivity for undiluted samples corresponds to 34.4 ng/mL, because the serum samples are instructed to be diluted at 20-fold (1:20) before starting the assay.

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