AAV TAb ELISA Kit

Instructions

Description

Adeno-associated viruses (AAVs) are non-pathogenic viruses that are commonly used in gene therapeutics as delivery vehicles. Natural immunization with Wildtype AAVs is common, and the presence of anti-AAV antibodies has been extensively reported both in humans and research animal models. Pre-existing immunity against AAV therapeutics presents a challenge for the efficacy of gene therapies, and evaluating the presence of anti-AAV antibodies is common in research and preclinical settings, as it allows the selection of naïve animals to avoid study biases. The VRL AAV TAb ELISA Kit contains all the necessary material to test in house for the presence of anti-AAVs IgG antibodies in NHP serum.

The VRL TAb assay is based on the indirect ELISA technique. AAV virus-like particles (VLP) are used as capture antigen. The VLPs are immobilized onto a 96-well microplate. Specific IgG antibodies, directed against the AAV capsid present in the sample, would bind the VLPs. The captured antibodies are then detected with a secondary conjugated anti non-human primate antibody (detection antibody).

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Materials included	Required Material
• Coated 96-well plate • Positive and negative controls • Sample and reagent diluent • Detection antibody • Substrate • Stop solution	• Single and multichannel pipettes • Sterile pipette tips for different volumes • Eppendorf tubes • Distilled water • ELISA Plate Reader (450/650 nm)

Procedure

1. Remove all kit reagents and plates from storage and let warm up to room temperature (RT, 20 – 26°C). Remove plate seal by gently peeling off the film
2. Dilute sample each sample and control 1/100 in Diluent (3 µL of Sample in 297µL of diluent)
3. Add 100 µL of each sample and controls to the plate (Refer to recommended template provided)
4. Incubate at room temperature for 1 hour
5. Wash 3 times with 200 µL of diluent and tap the plate to evacuate all liquid
6. Add 100 µL of Detection Antibody and incubate 1 hour at room temperature
7. Wash 3 times with 200 µL of diluent and tap the plate to evacuate all liquid
8. Add 100 µL of Substrate and incubate for 10 minutes (+/- 1 min) at RT
9. Add 100 µL of STOP solution
10. Read the plate immediately in a standard plate reader.

Note: This assay produces a yellow pigment that can be read (optical density or OD) at 450 nm in any standard plate reader. Optical subtraction (sometimes called correction) at 630 nm is recommended.

Results calculations

1. Negative controls OD (450 nm) value should range from 0.00 to 0.299
2. Positive controls OD (450 nm) should be 0.80 to 3.00
3. Samples OD values over 0.5 are considered positive
4. If any OD value for the negative/positive controls fall out of the ranges described above, the test is considered invalid and a re-test would be necessary.