Anti-AAV2 Antibody ELISA Kit for Monkey

Code No. 5123/5124

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Intended Use

Anti-AAV2 Antibody ELISA Kit for Monkey can be used to measure monkey anti-AAV2 antibodies in sera.
To measure monkey anti-AAV2 antibody, please use two Anti-AAV2 Antibody ELISA Kits for Monkey (MBL; Code No. 5123 and 5124) in combination. The two kits are sufficient to produce one 96-well microplate.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store #5123 components at -80°C, and #5124 components at 2-8°C.
- Don't expose reagents to excessive light.

Materials Provided

Code No. 5123 (store at -80°C)

Name	Materials	Quantity
AAV2 empty capsid	Antigen for solid phase to the Microplate	20 µL × 1 vial
Anti-AAV2 antibody standard	anti-AAV2 antibodies	200 µL × 1 vial

* The components should be stored at -80°C. Avoid repeated freeze-thaw cycles.

Code No. 5124 (store at 2-8°C)

Name	Materials	Quantity
Microplate	Microwell strips	8-well × 12 strips
Coating buffer	Buffer for coating microwells with AAV2 empty capsid (Ready-to-use)	20 mL × 1 bottle
Blocking buffer	Buffer for blocking microwells (Ready-to-use)	100 mL × 1 bottle
Sample diluent	Buffer for diluting samples (Ready-to-use)	30 mL × 1 bottle
Conjugate diluent	Buffer for diluting HRP conjugated antibody (Ready-to-use)	14 mL × 1 bottle
HRP conjugated antibody	HRP conjugated anti-IgG (Monkey) polyclonal antibody (100x)	150 µL × 1 vial
Wash concentrate (20x)	Buffer for washing microwells (20x)	50 mL × 1 bottle
Substrate reagent	TMB/H_2O_2 solution (Ready-to-use)	20 mL × 1 bottle
Stop solution	0.5N H_2SO_4 solution (0.25 M) (Ready-to-use)	20 mL × 1 bottle
Plate seals	Plate seals	3 pieces

Materials Required but not Provided

- **Pipettors (single and multichannel):** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Plastic tubes** (1.5 mL, 15 mL etc.)
- **(Optional) Microplate washer:** Manual washing is possible.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**
- **Plate reader:** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/620 nm. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**

Precautions and Recommendations

- All reagents need to be brought to room temperature (18-27°C) before use.
- Do not use kit components beyond the indicated kit expiration date.
- Do not mix reagents with different batches and kits.
- Do not mouth pipette or ingest any of the reagents.
- Fresh samples should be used. Aliquot each sample and store below -20°C if necessary. Avoid repeated freezing and thawing. Never store the samples at 4°C, as samples might be affected by storage at this temperature.
- AAV2 empty capsid is easy to adsorb on polystyrene. When handling AAV2 empty capsid, please use polypropylene tubes and tips.
- The buffers and reagents in this kit may contain preservatives. Care should be taken to avoid direct contact with these reagents. During disposal, flush with plenty of water and/or handle it according to the regulations of the facility.
- Dispose tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic **Stop solution** and **Substrate reagent**, which contains hydrogen peroxide. Protect eyes and skin and handle with care. In case of contact with the **Stop solution** and the **Substrate reagent**, wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and eye protection when handling immunoassay materials and samples of human/monkey origin, and these reagents.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **AAV2 empty capsid is a kit component subject to the regulations of the Cartagena, Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms. Please handle carefully according to the regulations of each user's facility.**
- **AAV2 empty capsid is known as non-infectious against human, however, instruments used in this assay should be treated according to the regulations of each facility after the assay.**
  (e.g.)
  Soak in 2% glutaraldehyde solution (final concentration) for more than one hour.
  Soak in 0.1% sodium hypochlorite solution (available chloric: approximately 1,000 ppm.) for more than one hour.
  Autoclave at 121°C for more than 20 minutes.

Sample Collection and Storage

Serum: Use a serum separation tube and separate the serum according to the manufacturer's manual. Fresh samples should be used. Aliquots of serum may also be stored at below -20°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Other biological samples: Not validated by MBL.

Summary of Procedure

To prepare the antigen-**coated** wells: Add 100 µL of AAV2 Antigen Solution to the wells

To prepare the antigen-**UNCOATED** wells: Add 100 µL of Coating buffer to the wells

↓ Incubate over night at 2-8°C.

Wash the wells with Blocking buffer

↓

Add 200 µL of Blocking buffer to the wells

↓ Incubate for 2 hours at room temperature (18-27°C).

Add 100 µL of standards and samples to the wells

↓ Incubate for 2 hours at room temperature (18-27°C).

Wash the wells

↓

Add 100 µL of Conjugate Solution

↓ Incubate for 1 hour at room temperature (18-27°C).

Wash the wells

↓

Add 100 µL of Substrate reagent

↓ Incubate for 20 minutes at room temperature (18-27°C).

Add 100 µL of Stop solution

↓

Measure absorbance at 450 nm

Detailed Protocol

The **Anti-AAV2 Antibody ELISA Kit for Monkey** is provided with removable strips of wells so the assay can be carried out on separate occasions. Experimental conditions may vary. To measure monkey anti-AAV2 antibody, please use two Anti-AAV2 Antibody ELISA Kits for Monkey (MBL; Code No. 5123 and 5124) in combination. The two kits are sufficient to produce one 96-well microplate. All samples and the positive control should be assayed in duplicate. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

<Preparation of Working Solutions>

All reagents need to be brought to room temperature prior to assay. **Sample diluent** should be inverted and mixed before use.

1. Wash Buffer
   Prepare "Wash Buffer" by adding 50 mL of the **Wash concentrate (20x)** to 950 mL of deionized (distilled) water (ddH_2O). Mix well.

2. AAV2 Antigen Solution
   The "AAV2 Antigen Solution" should be used immediately after preparation. Prepare "AAV2 Antigen Solution" by diluting **AAV2 empty capsid** with **Coating buffer** according to the attached lot specific document "*Preparation of Antigen*". **AAV2 empty capsid** should be thawed at room temperature (18-27°C), and mixed gently. Do not vortex strongly.
   *__**AAV2 empty capsid** is easy to adsorb on polystyrene. When handling **AAV2 empty capsid**, please use polypropylene tubes and tips.__

3. Anti-AAV2 Antibody Standard Solution (Master Standard)
   Prepare "Anti-AAV2 Antibody Standard Solution" by diluting **Anti-AAV2 antibody standard, 25-fold** with **Sample diluent** by gently mixing (*e. g.*, add 40 µL of **Anti-AAV2 antibody standard** to 960 µL of **Sample diluent**). After thawing **Anti-AAV2 antibody standard**, dispense it in small aliquots (*e.g.* 50 µL) to plastic micro-centrifuge tubes and store below -80°C to avoid non-specific adsorption to glass surface and multiple freeze-thaw cycles.
   The prepared Anti-AAV2 Antibody Standard Solution is referred to as the Master Standard.

   * Sample diluent should be inverted and mixed before use.

   Prepare Standard dilution series as follows:
   Use the Master Standard to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The Master Standard (Std.1) serves as the highest standard.

	Volume of Standard	Sample diluent	Dilution ratio
Std. 1	1,000 µL of Master Standard	0 µL	x25 (100 U/mL)
Std. 2	500 µL of Std. 1	500 µL	x50 (50 U/mL)
Std. 3	500 µL of Std. 2	500 µL	x100 (25 U/mL)
Std. 4	500 µL of Std. 3	500 µL	x200 (12.5 U/mL)
Std. 5	500 µL of Std. 4	500 µL	x400 (6.25 U/mL)
Std. 6	500 µL of Std. 5	500 µL	x800 (3.125 U/mL)

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Sample diluent before dispensing. The Sample diluent serves as the Blank.

4. Conjugate Solution

Prepare "Conjugate Solution" by diluting **HRP conjugated antibody, 100-fold** with **Conjugate diluent**. Prepare only a sufficient amount of the Conjugate Solution immediately before use (*e. g.*, add 100 µL of **HRP conjugated antibody** to 9,900 µL of **Conjugate diluent**).

5. Other reagents are ready-to-use.

<Preparation of samples>

Samples require the proper dilution ratio if necessary. As an example, serum samples used in MBL were diluted 20-fold with **Sample diluent**. If the measured value falls outside the range of the calibration curve, we recommend verifying the dilution ratio and retesting. (*e. g.*, add 20 µL of each sample to 380 µL of **Sample diluent**)

* Sample diluent should be inverted and mixed before use.

<Assay Procedure>

Coating

***Please note that both antigen-coated wells and antigen-UNCOATED wells should be prepared for the assay.**

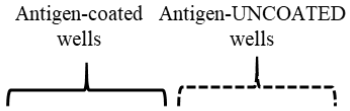
1. To prepare the antigen-coated wells, pipette **100 µL** of AAV2 Antigen Solution to the wells of **Microplate** with a multichannel pipet.

2. To prepare the antigen-UNCOATED wells, pipette **100 µL** of Coating buffer to the wells of **Microplate** with a multichannel pipet.

3. Incubate the plate overnight at 2-8°C. Please be careful not to evaporate the solution in the wells.

4. Discard the solution in the wells, and wash the plate twice by filling each well with **Blocking buffer** (200 µL) using a multichannel pipette. Tap the plate on a paper towel to remove any remaining solution.
   *Wash as quickly as possible, do NOT let wells dry up.

An example of using Microwell strips;



Std.1	Std.1	Std.1	Std.1	S2	S2	S2	S2	S10	S10	S10	S10
Std.2	Std.2	Std.2	Std.2	S3	S3	S3	S3	S11	S11	S11	S11
Std.3	Std.3	Std.3	Std.3	S4	S4	S4	S4	S12	S12	S12	S12
Std.4	Std.4	Std.4	Std.4	S5	S5	S5	S5	S13	S13	S13	S13
Std.5	Std.5	Std.5	Std.5	S6	S6	S6	S6	S14	S14	S14	S14
Std.6	Std.6	Std.6	Std.6	S7	S7	S7	S7	S15	S15	S15	S15
Blank	Blank	Blank	Blank	S8	S8	S8	S8	S16	S16	S16	S16
S1	S1	S1	S1	S9	S9	S9	S9	S17	S17	S17	S17

Blocking

5. Pipette **200 µL** of **Blocking buffer** to the wells with a multichannel pipet.

6. Incubate the plate for 2 hours at room temperature (18-27°C).

7. Discard the solution in the wells, and tap the plate on a paper towel to remove any remaining solution.

1st Reaction

8. Pipette **100 µL** of prepared Standards and diluted samples to the wells, both antigen-coated wells and antigen-UNCOATED wells respectively.

9. Incubate the plate for 2 hours at room temperature (18-27°C).

Washing

10. Discard the solution in the wells.

11. Wash the plate 4 times by filling each well with Wash Buffer using a squirt bottle, multichannel pipette, manifold dispenser or microplate washer. Tap the plate on a paper towel to remove any remaining solution.
    * When using an auto-washer, appropriate washing times may vary depending on the instrument.

2nd Reaction

12. Pipette **100 µL** of Conjugate Solution to the wells with a multichannel pipet.

13. Incubate the plate for 1 hour at room temperature (18-27°C).

Washing

14. Discard the solution in the wells.

15. Wash the plate 4 times as in step 11.

Enzyme Reaction

16. Pipette **100 µL** of **Substrate reagent** to the wells with a multichannel pipet.

17. Incubate the plate for 20 minutes at room temperature (18-27°C).

Reading

18. Pipette **100 µL** of **Stop solution** to the wells with a multichannel pipet.

19. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/620 nm. The primary wavelength is 450 nm, and the reference wavelength is 620 nm. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop solution.

Note-1: Each solution and reagent should be used at room temperature (18-27°C).
Note-2: Use new disposable tips, reservoirs and paper towels at each step to avoid contamination.
Note-3: Once the solution has been poured into the reservoir, never return it to the bottle.
Note-4: Complete removal of liquid at each step is essential to good performance.
Note-5: Ensure that the back of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading.
Note-6: The incubation time for color development may vary depending on the environment such as temperature. It can be adjusted according to the coloring intensity.

Calculations

Anti-AAV2 antibody concentration in each sample can be calculated using a 4-parameter logistic regression equation.

1) Calculate SPEC O.D. for each well
(O.D. value of antigen-coated wells) – (O.D. value of antigen-UNCOATED wells) = SPEC O.D.

2) Plot the SPEC O.D. values average of the duplicate measurements for each standard except Blank wells (Y) versus the known concentration (X) of each standard, and draw the best fit curve. Most microtiter plate readers perform automatic calculations of analyte concentration. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration (U/mL).

Measurement Range

The measurement range is 3.1 U/mL to 100 U/mL. Any sample reading higher than the highest standard should be diluted with Sample diluent in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the concentration of the sample.

Quality Control

To assure the validity of the results, each assay must include both Blank and the highest standard. The net O.D. values (450 nm) of these controls must fall within the ranges listed below. If O.D. values do not meet the requirements, the assay is invalid and requires repeat of the assay.

O.D. values of Blank ≤ 0.09
SPEC O.D. values of the highest standard (Std. 1) ≥ 1.0

Troubleshooting

1. All samples and Blank should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. Do not dry the plate as it can adversely affect the assay results. Immediately add each solution step by step.

Reagent Stability

#5123 components must be stored at -80°C, and #5124 components must be stored at 2-8°C. Reagents should not be used beyond the stated expiration date.

Assay Characteristics

1. Precision

Intra-assay Precision (Precision within an assay)
Samples of known concentration were tested in sextuplicate on one plate to assess intra-assay precision.

• Intra-assay (Within-Run, n=6)

	Sample 1	Sample 2	Sample 3
1	70.0	42.9	17.4
2	65.6	42.8	15.6
3	68.5	42.4	16.6
4	68.5	44.8	16.4
5	67.6	44.1	15.6
6	66.2	45.2	14.7
Max.	70.0	45.2	17.4
Min.	65.6	42.4	14.7
Mean	67.7	43.7	16.1
SD	1.6	1.2	0.9
CV (%)	2.4	2.7	5.9

Inter-assay Precision (Precision between assays)
Three sextuplicate-measurements were performed to assess inter-assay precision. Samples of known concentration were used.

• Inter-assay (Run-to-Run, n=3)

	Sample 1	Sample 2	Sample 3
1	67.7	43.7	16.1
2	61.6	45.3	14.8
3	79.5	41.1	15.3
Mean	69.6	43.4	15.4
SD	9.1	2.1	0.7
CV (%)	13.1	4.9	4.3

Examples of Test Results

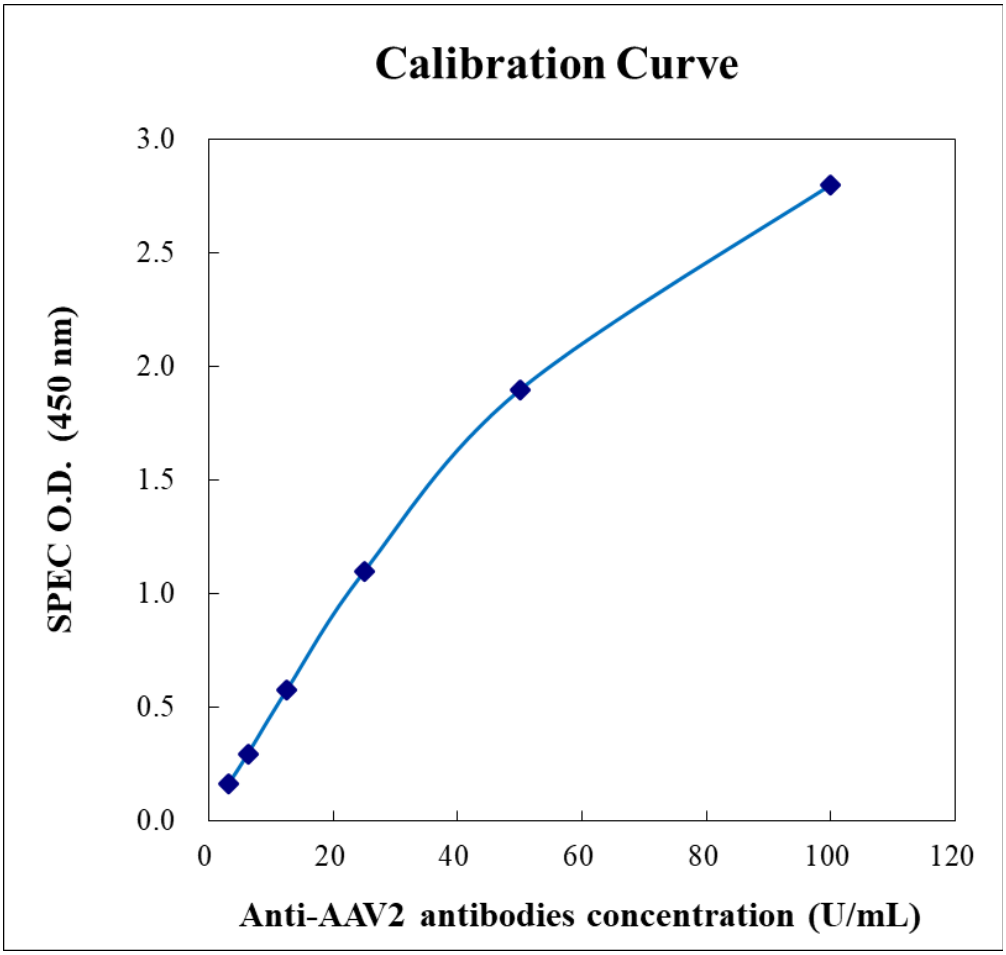


Fig.1 Anti-AAV2 antibodies calibration curve

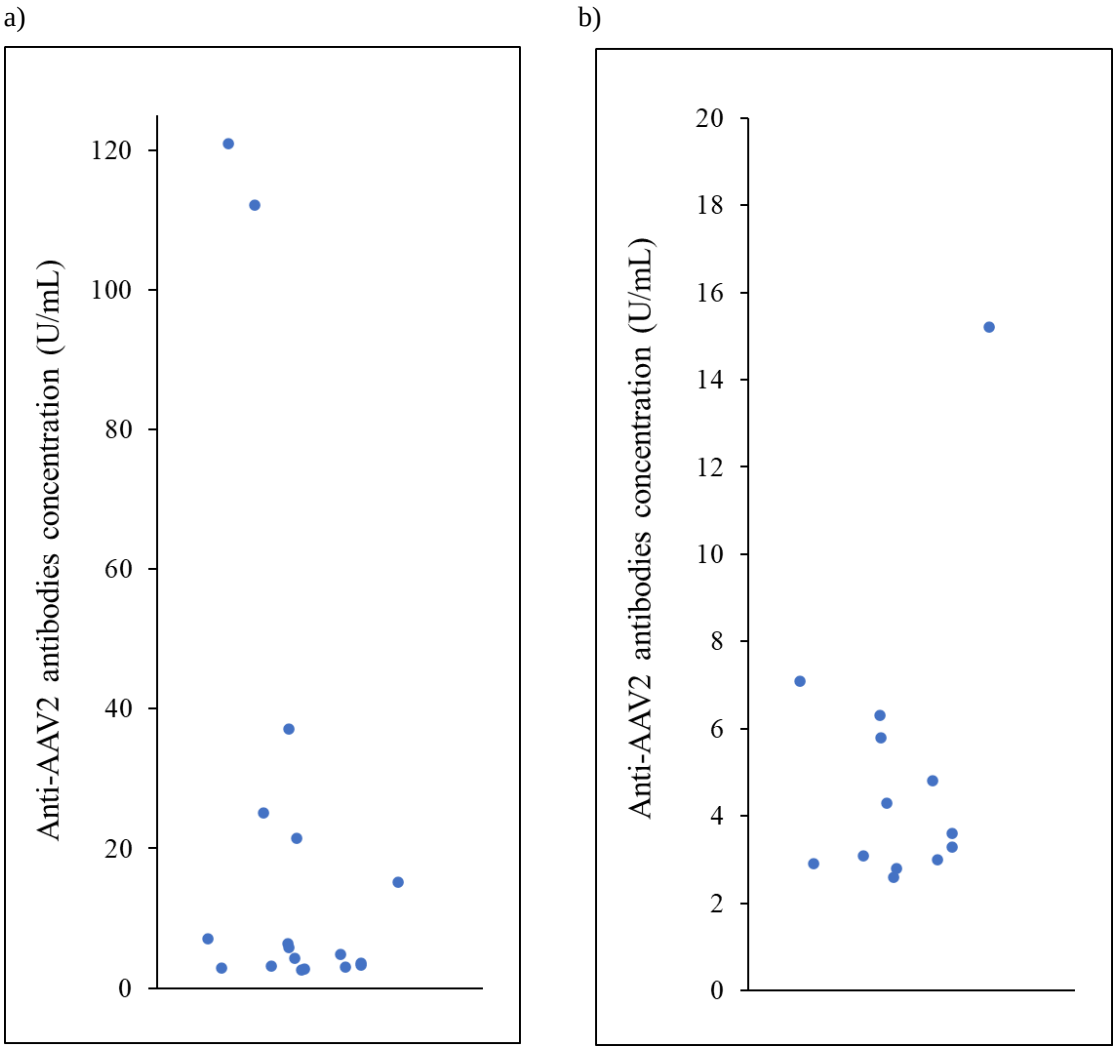


Fig.2 The Anti-AAV2 antibody concentration serum derived from Monkey (n=18). These samples were diluted 20-fold with Sample diluent.
a) A figure showing distribution of measured values of 18 samples.
b) An enlarged figure of the plot of less than 20 U/mL. (13 out of 18 samples)

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Preparation of Antigen

Lot No. XXXXXX

To prepare "AAV2 Antigen Solution" by diluting **AAV2 empty capsid** with Coating buffer.

1. Thaw **AAV2 empty capsid** at room temperature (18-27°C).
2. Dilute the thawed **AAV2 empty capsid** **X-fold** with Coating buffer.
   (e. g., Add the thawed **Y µL** of **AAV2 empty capsid** to **Z µL** of Coating buffer)

* **AAV2 empty capsid** should be mixed with Coating buffer immediately. The "AAV2 Antigen Solution" should be used immediately after preparation.

* **AAV2 empty capsid** is easy to adsorb on polystyrene. When handling **AAV2 empty capsid**, please use polypropylene tubes and tips.