



# AAV6 Human Antibody ELISA (IgG) Manual

Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of human anti-AAV6 IgG in human serum or plasma.

<b>Catalog No.:</b>	PR5406
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<b>Contents:</b>	12 x 8 Determinations
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<b>Storage conditions:</b>	2–8°C
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<b>Version:</b>	02
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For research use only.

**PROGEN**



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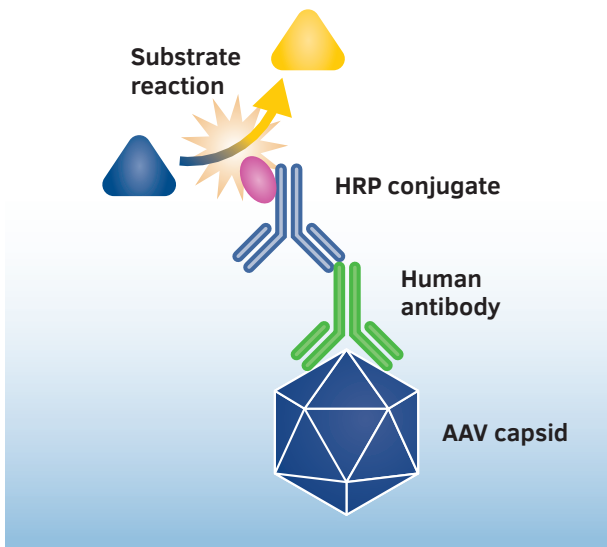
# 1. Introduction

Recombinant adeno-associated virus (AAV) vectors are leading tools for viral gene therapy. However, many humans in the general population have developed antibodies against AAV as a result of naturally acquired infections, which might affect efficacy and safety of the gene transfer using AAV vectors. For a safe and efficient gene therapy, it is indispensable to screen patients for these pre-existing AAV antibodies. Furthermore, AAV total antibody levels due to natural infections compared to those after AAV gene therapy might differ. Therefore, it is also important to monitor the kinetics of AAV antibody levels after natural infections and after systemic AAV gene therapy treatment.

PROGEN's AAV6 human antibody ELISA (enzyme-linked immunosorbent assay) offers a fast, sensitive and reproducible tool for quantifying these pre-existing AAV antibodies [immunoglobulin G (IgG)] in human sera. The ELISA is developed to determine total anti-AAV IgGs, including neutralizing antibodies (NABs) and non-neutralizing antibodies (nNABs).

## 2. Test Principle

The assay is based on the ELISA technique (*see figure below*). Recombinant fully assembled and intact empty AAV6 capsids are immobilized onto wells of a microplate. Human antibodies specific to AAV6, that are present in the sample, bind to the AAV6 capsids. The captured AAV6 human antibodies are then detected in a two-step process.



HRP-coupled anti-human IgG antibodies specifically bind the human IgG antibodies from the human sample. After adding the substrate, the conjugated peroxidase catalyzes the substrate resulting in a color reaction. The absorbance is measured photometrically at 450 nm (optional: reference wavelength at 650 nm) and is proportional to the amount of specifically bound IgG antibodies to AAV6 capsids in the sample. The provided standard is a human monoclonal antibody specific to fully assembled and intact AAV6 capsids, which results in a standard curve, allowing for the quantitative determination of samples in arbitrary units.

The provided positive control consists of a human serum, which was tested positive for anti-AAV6 IgG antibodies. The provided negative control consists of a human serum, which does not contain anti-AAV6 IgG antibodies.

### 3. Required Material

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Precision pipettes

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Sterile pipette tips

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Distilled water

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Reaction tubes

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ELISA Reader (450 nm, optional: reference wavelength at 650 nm)

## 4. Test Kit Contents

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<b>AAV6 microplate</b>	12 x 8-well-strips, coated with AAV6 empty capsids in re-sealable aluminum bag with desiccant, 1 plate. Ready-to-use.
<b>IgG standard 1 (16 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.
<b>IgG standard 2 (8 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.
<b>IgG standard 3 (4 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.
<b>IgG standard 4 (2 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.
<b>IgG standard 5 (1 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.
<b>IgG standard 6 (0.5 U)</b>	anti-AAV6 human monoclonal recombinant antibody, 1 x 0.5 ml. Ready-to-use.

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<b>Positive control</b>	1 x 0.5 ml. Ready-to-use.
<b>Negative control</b>	1 x 0.5 ml. Ready-to-use.
<b>Buffer B01 (20x)</b>	2 x 20 ml. Dilute before use.
<b>HRP conjugate (100x)</b>	anti-AAV6 human IgG, 1 x 0.125 ml. Dilute before use.
<b>TMB</b>	Substrate, TMB (tetramethylbenzidine), 12 ml. Ready-to-use.
<b>STOP</b>	Stop Solution, 13 ml. Ready-to-use.
<b>Adhesive foil</b>	2 pieces.

## 5. Preparation of Reagents

### Buffer B01 (20x)

The buffer concentrate may contain salt crystals, which can be dissolved by rolling the bottle on a roller mixer at room temperature or by placing the bottle shortly at 37°C (e.g., in a water-bath, let buffer cool down to RT before use).

Preparation and pre-dilution of Buffer B01 (20x):  
Dilute required reagent volumes immediately before use.

1. Dilute 1:20 with distilled water.
2. The diluted component is named Buffer B01 (1x).

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## **HRP conjugate (100x)**

Let the HRP conjugate (100x) reach room temperature.

Preparation and pre-dilution of HRP conjugate (100x):  
Dilute required reagent volumes immediately before use.

1. Dilute 1:100 with Buffer B01 (1x).
2. The diluted component is named HRP conjugate (1x).

## 6. Sample Preparation Before Use

Allow samples to reach room temperature prior to assay. Take care to mix test samples gently in order to ensure homogeneity. Standards, positive control and negative control are ready-to-use and do not have to be diluted.

**The test samples have to be diluted immediately before use.** Pre-dilute your sample 1:100 in Buffer B01 (1x). Depending on the results (see 10. Calculation of results), retesting or further dilution of the sample may be necessary. If high antibody concentrations are expected, multiple dilutions might be needed.


### Example for a plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Blank	Blank								
B	Std2	Std2	Sp1	Sp1								
C	Std3	Std3	Sp2	Sp2								
D	Std4	Std4	Etc.	Etc.								
E	Std5	Std5										
F	Std6	Std6										
G	PC	PC										
H	NC	NC										

Std: standard; PC: positive control; NC: negative control; Sp: sample; Blank: Buffer B01 (1x)

## 7. Short Protocol

**1** Sample dilutions  
Standards  
Controls




100 µl

Incubate  
30 min  
at RT

 3 times 200 µl Buffer B01 (1x)

**2** HRP conjugate  
(1x)

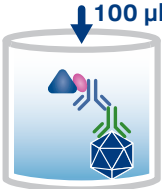


100 µl

Incubate  
30 min  
at RT

 3 times 200 µl Buffer B01 (1x)

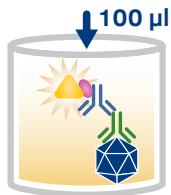
**3** TMB



100 µl

Incubate  
15 min  
at RT

**4** Stop



RT

**5** Read at 450 nm  
(and 650 nm)



Read  
within  
30 min

## 8. Test Procedure

Prior to use, allow kit reagents to reach room temperature (RT, 20–26°C). **Do not shake the plate during the incubation steps.**

1. Pipette 100 µl of each, IgG standards, positive control, negative control and the pre-diluted samples [in **Buffer B01 (1x)**] into the corresponding wells of the microplate strips. Duplicates are recommended. Seal strips with adhesive foil and incubate for **30 min at RT**.

Discard content of microtiter strips. For washing, pipette 200 µl of **Buffer B01 (1x)** into each well, incubate approximately 5 sec, discard and tap inverted plate onto absorbent paper. Carry out **three** washing steps in total.

2. Pipette 100 µl of the pre-diluted **HRP conjugate (1x)** into each well. Seal strips with adhesive foil and incubate for **30 min at RT**.

Repeat washing as described in step 1.

3. Pipette 100 µl of ready-to-use **TMB** into each well. Seal strips with adhesive foil and incubate for **15 min at RT**.

4. Stop color reaction by adding 100  $\mu$ l of **STOP** into each well.
5. Make sure no air bubbles are in the wells. **Within 30 min**, measure color intensity with a photometer at a wavelength of 450 nm (optional: reference wavelength at 650 nm).

## 9. Calculation of Results

If applicable, subtract values measured at 650 nm reference wavelength from values at 450 nm.

Calculate the average absorbance values for each duplicate set of standards and specimen dilutions. Create a standard curve by plotting the mean absorbance value of each standard (y-axis, linear scale) against the corresponding units (x-axis, logarithmic scale recommended).

Use a best fit curve for calculating the results. We suggest using a suitable computer program for the calculation. A 4-parameter logistic fit (4PL) is recommended.



### **Please note:**

The standard curve needs to be determined for each experiment individually.

For this assay, cut-off determination was performed using 1:100 serum dilutions.

Thus, for samples diluted 1:100 and ready-to-use controls, the units read from the standard curve already correspond to the final result.

If a sample must be diluted further because its titer is outside the linear range, an additional dilution factor must be applied.

The dilution factor is calculated relative to the standard 1:100 dilution. Multiply the value obtained from the standard curve by this dilution factor to determine the amount of units in the sample.

**Table 1. Examples for determining the dilution factor based on the test sample dilution**

Final Sample Dilution	Dilution Factor	Calculation
1:100	1	Use the value [units] directly from the curve
1:200	2	Multiply units $\times 2$
1:500	5	Multiply units $\times 5$
1:1000	10	Multiply units $\times 10$

## Interpretation of results

The kit is quantitative over the whole range of standard the standard curve. For highest accuracy, the OD values of unknown samples should ideally be in the recommended minimal range for quantification\*:

0.30 units – 6.80 units

\* The minimal linear detection range [units] was determined by titrating three different serum samples serotype and identifying the consistent linear range.

The definition of the cut-off included the analysis of 100 randomly selected serum samples from healthy blood donors in Europe using our five different human IgG antibody ELISAs (AAV2, AAV5, AAV6, AAV8 and AAV9). To determine the technical cut-off value of the assay, the background noise was assessed using uncoated microplates. The background level plus three standard deviations was used as a threshold to differentiate AAV-seropositive from AAV-seronegative samples, resulting in a final cut-off value of 1.1 units for AAV-seropositivity.

The biological cut-off may need to be determined individually, depending on the specific biological or clinical context.

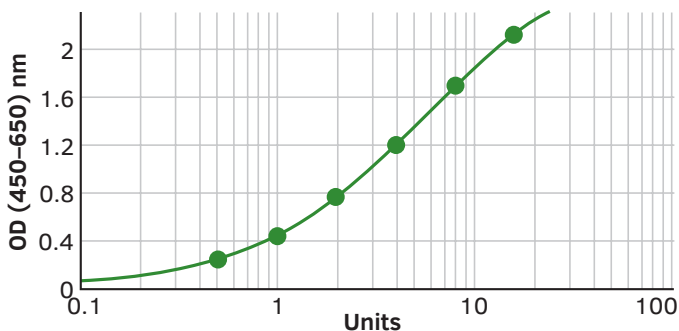
Negative: <0.9 units

Indeterminate: 0.9 – 1.1 units

Positive: >1.1 units

\*For indeterminate results, retesting may be necessary.

### Example for a standard curve:



## 10. Test Validity

The test validity parameters are stated on the Quality Control Certificate included in each kit.

## 11. Storage & Stability

Storage for all kit components: 2–8°C until indicated expiry date.

Stability after opening: 4 weeks at 2–8°C: Buffer B01 (1x).

## 12. General Information

**For professional use.**

### **Release notes**

The instruction manual is only valid in combination with the lot-specific documents (Quality Control Certificate), which are enclosed in each kit.

Please make sure to use the instruction manual with the version number that corresponds to the number on the lot-specific documents.

## Precautions

All liquid components except TMB and STOP contain a preservative. Do not swallow. Avoid any contact with skin or mucous epithelia!

STOP (sulphuric acid) and TMB may cause skin or eye irritation. In the event of eye contact, rinse out immediately with plenty of water and consult a physician!

The provided sera (positive and negative control) were tested and found negative for HBsAg and HIV as well as for HCV antibodies. Nonetheless, handle all components and all samples as if potentially hazardous.

Safety data sheet is available on request.

## Disposal

**Product:** Chemicals and biological materials must be disposed of in compliance with the respective national regulations.

**Packaging:** Packaging must be disposed of in compliance with the respective national regulations. Handle contam-

inated packaging in the same way as the product itself. If not officially specified otherwise, non-contaminated packaging may be treated like household waste or may be recycled.

## **Transport damages**

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use damaged components for test procedure. Such components or kits should be stored at 2 – 8°C until the complaint is handled.







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