



# Dip'n'Check AAV1 and AAV6 Manual

<b>Cat. No.:</b>	PR5216
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<b>Contents:</b>	25 lateral flow tests
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<b>Storage box 1/2:</b>	2 – 8°C
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<b>Storage box 2/2:</b>	Up to 8 weeks 2 – 8°C Long-term -20°C
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<b>Version:</b>	03
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# 1. Introduction

Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses, which are subject of intense studies as viral vectors for gene therapy. The virus transduces a variety of dividing and non-dividing cells showing long-term gene expression with low cellular immune response.

Methods for the characterization of AAV preparations currently include titration ELISA, qPCR, ddPCR, DNA dot blot, cell based assays, SDS-PAGE or electron microscopy.

PROGEN's Dip'n'Check AAV1 and AAV6 is a lateral flow sandwich assay for the detection of fully assembled AAV1 or AAV6 capsids using an antibody specific for AAV1 and AAV6. The quick and reliable detection of AAV samples allows a rapid estimation and comparison of concentrations in various samples for further downstream analysis or purification steps (e.g. for finding the optimal dilution range for subsequent ELISA analysis).

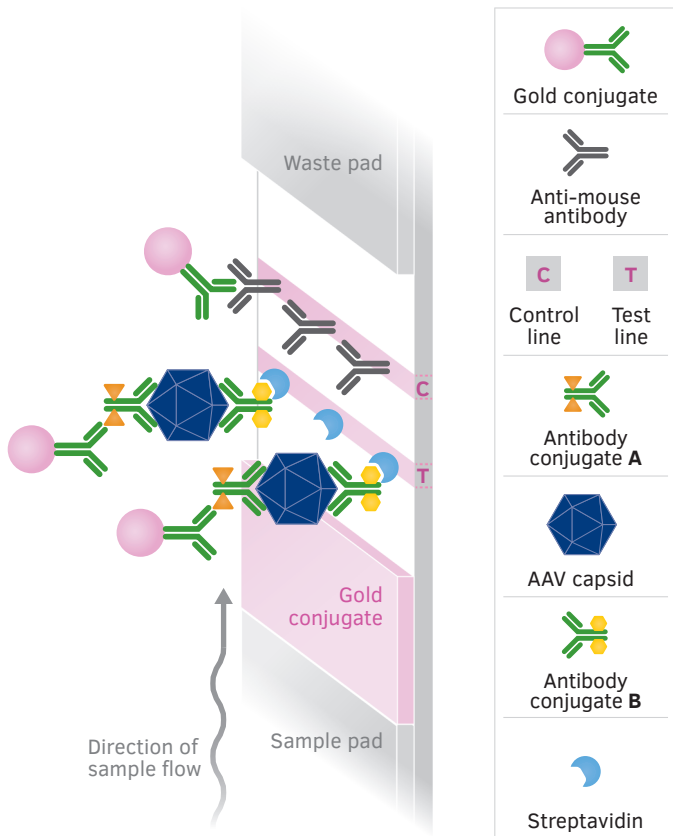
This lateral flow test is a semi-quantitative assay with a detection range of **1.0E+08 – 1.0E+10 capsids of AAV1 and AAV6** in the final assay volume of **156 µl**. Within this range, a 2-fold concentration difference is detectable.

## 2. Test Principle

The test is based on the specific binding of an anti-AAV1/AAV6 antibody to fully assembled viral capsids. Empty and full capsids show no difference in binding to this antibody. The antibody is coupled with two different conjugates, antibody conjugate A and B. During the test run, conjugate B binds to the immobilized streptavidin on the test line, while conjugate A is captured by a conjugate-specific antibody coupled to gold nanoparticles to visualize the AAV-antibody-complex at the test line. Further, the gold-conjugate binds to specific anti-mouse antibodies located at the control line (*Figure 1*).

Both AAV antibodies delivered with this test are mixed in equal volumes with running buffer and the AAV sample, and incubated for 10 min to allow binding of antibodies to the capsids. Ideally, the AAV sample should be tested in different dilutions. Then, the test strip is placed in this solution to allow complex formation with the gold nanoparticles and upward migration of the solution by capillary force to the test and control lines.

**Figure 1:**  
**Test principle of the Dip'n'Check AAV1 and AAV6**



## Note:

1. The control line's intensity is generally constant over the detection range of this test and should always appear as a strong purple line. However, very high concentrations of capsids above the detection range of this test (i.e. titer  $>1.0E+10$  capsids) can reduce the amount of available gold particles and thus lead to a weaker or absent control line. In such cases, the test should be repeated with a higher dilution of the sample (e.g. 5- to 10-fold).
2. The **detection range** of  **$1.0E+08$ – $1.0E+10$**  capsids of AAV1 or AAV6 in the assay volume (156  $\mu$ l) corresponds to  $1.0E+09$ – $1.0E+11$  capsids/ml in the diluted AAV sample of 100  $\mu$ l.

### 3. Required Material

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Precision pipets

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Pipet tips

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Phosphate buffered saline (PBS) and reaction tubes for dilution of the AAV sample, if necessary

### 4. Test Kit Contents

#### Box 1/2

<b>AAV test strips</b>	25 lateral flow test strips in a plastic container
<b>Running buffer</b>	1 x 1.5 ml, ready-to-use
<b>Reaction tubes</b>	25 x 2 ml

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#### Box 2/2

<b>Antibody conjugates A and B</b>	100 µl each
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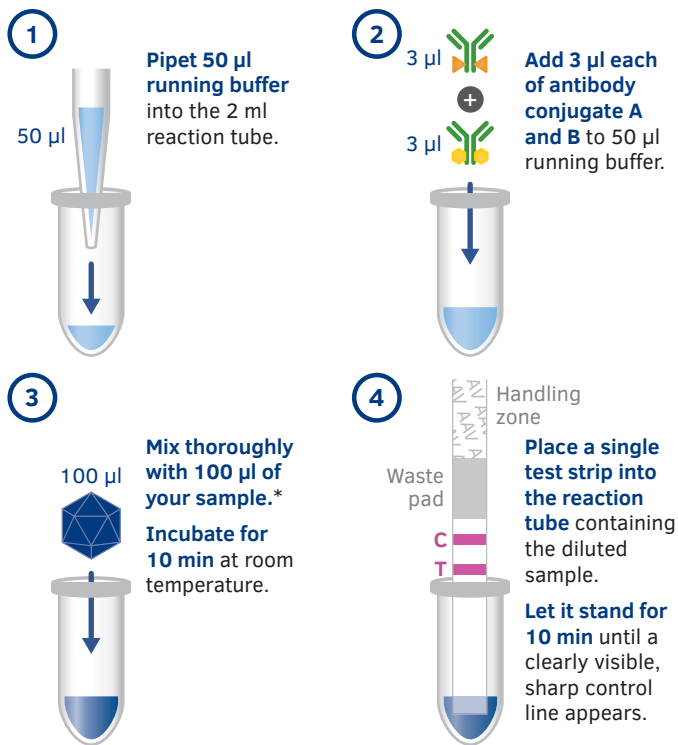
## 5. Preparation of Reagents

Prior to use, it is important to allow the AAV test strips to reach room temperature (RT, 20–26°C) in order to avoid condensation of moisture on the test strip. After taking out the required number of strips, immediately **close the plastic container** and avoid longer exposure of the strips to moisture and light. Please **use gloves** and **only touch the handling zone** at the top end above the waste pad (*Figure 2*).

It is possible to test **crude lysates of HEK293 or insect cells**, no matrix effects were detected in samples with a total protein concentration of 100 µg/ml in the final test volume. Other additives frequently used in buffers have also been tested. The tolerated concentrations for these compounds are listed in **Table 1 of section 10 (Matrix effects)**.

## Figure 2: Dip'n'Check AAV1 and AAV6 test procedure

### Sample preparation



\*Use more than one dilution of your sample of cell lysate or purified AAVs, e.g. in PBS, to reach the detection range of this test.

## 6. Storage & Stability

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**Store box 1/2 at 2–8°C** until expiry date.  
Do not store the test strips in a freezer.

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**Store box 2/2 (antibody conjugates A and B) at 2–8°C for up to 8 weeks and for long term until expiry date at -20°C**, avoid more than 5 freeze/thaw cycles.

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### **Stability after opening**

Test strips: 8 weeks at 2–8°C.

## 7. Documentation of Results

The optical analysis of the test strips can be done within 20 minutes after incubation of the test strips. Simple comparison of the test lines by eye will provide sufficient accuracy to estimate the concentration range and differences between samples.

If available, a reader for lateral flow tests can be used to scan the strips for documentation or a more detailed analysis.

Alternatively, a camera or office scanner can be used to scan multiple strips in parallel taped on a sheet of paper.

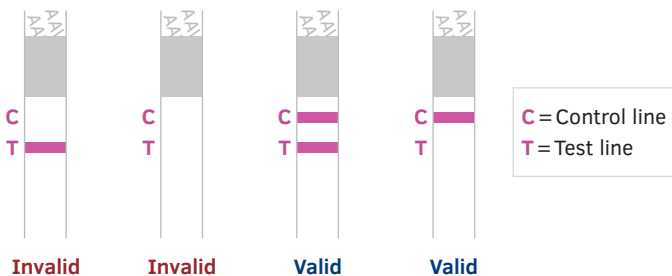
Further analysis can be performed using an image analysis software to get semi-quantitative results for your samples.

## 8. Test Validity

The upper control line must be clearly visible in all cases (positive control, negative control, unknown samples) in order to confirm the migration of gold particle complexes over the whole detection area of the strip.

If the staining of the control line is very weak or absent, the test is invalid and should be repeated with a higher dilution of the sample as mentioned above (*page 5*).

**Figure 3:**  
**Possible results of Dip'n'Check AAV1 and AAV6**



## 9. Test Characteristics

The detection range of the test strips is  $1.0E+08$  –  $1.0E+10$  capsids in the test volume for both AAV1 and AAV6.

## 10. Matrix Effects

Following additives (*Table 1*) were tested at the indicated concentration with no significant effect on the result of the lateral flow assay.

**Table 1:**  
**Concentrations of tolerated buffer additives on the Dip'n'Check AAV1 and AAV6**

Additive	Concentration
Pluronic F-68	0.1%
MgCl <sub>2</sub>	50 mM
Triton x-100	0.5%
Desoxycholate	0.05%
Tween 20	0.5%
EDTA	10 mM

Additive	Concentration
NaCl	500 mM
Iodixanol (Optiprep)	5%
Insect cell lysate	100 µg/ml
HEK293 cell lysate	100 µg/ml

## 11. General Information

### Transport conditions

Dip'n'Check AAV kits ship at room temperature to allow environmentally friendly delivery. Components are tested and stable at room temperature for the duration of shipment.

### 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.

### Precautions

Liquid components contain preservatives. Do not swallow! Avoid any contact with skin or mucous membranes. Safety data sheet available on request.

### Disposal

Products and packaging must be disposed of in compliance with the respective national regulations.





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