

How to use our AAV•Tek PCR Sample Prep Kit

To help you get optimal results, here's a step by step guide for our AAV•Tek™ PCR Sample Prep Kit.

The AAV•Tek PCR Sample Prep Kit allows for streamlined AAV quantification using a standardized method based on contemporary sample preparation methods. The kit consists of two key components needed for accurate and reproducible quantification of AAV titer levels: a DNase I Treatment and a Dilution Buffer. The DNase I Treatment includes all necessary reagents required to remove exogenous DNA surrounding the AAV capsid, while the Dilution Buffer prevents capsid aggregation and stabilizes genomic AAV DNA that is exposed when capsids are disrupted.

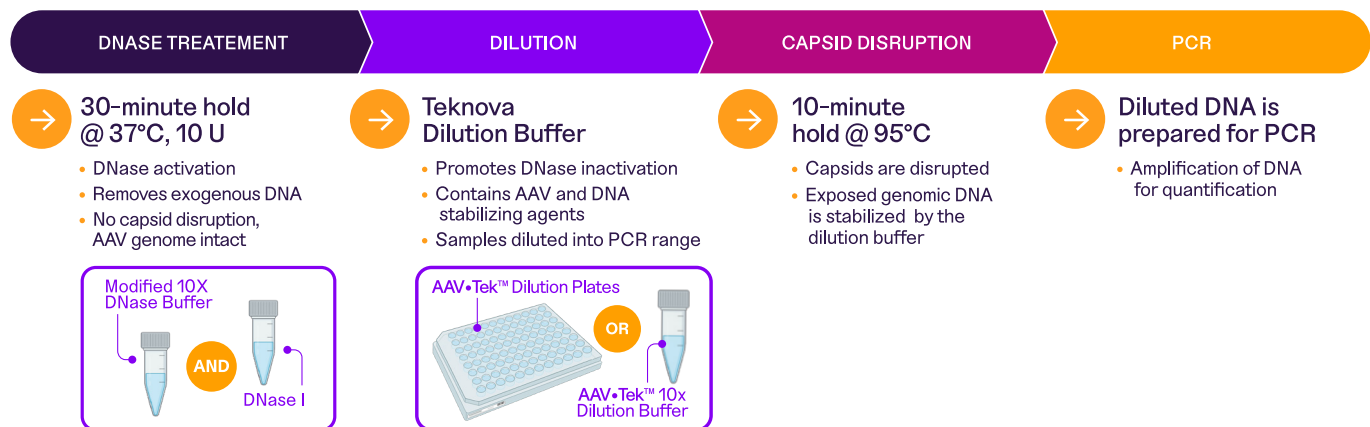
Purpose of the kit:

This kit is designed to promote accurate titers by addressing existing challenges within the sample preparation step to improve your AAV analytics. These include complete DNase inactivation, PCR inhibition, and irreproducible titers. Once you've used the kit, you can store your sample or transfer it to a separate tube or plate for either quantitative or digital PCR.



This protocol is applicable to following products:

For your convenience, we offer the kit with the Dilution Buffer either in pre-filled 96-well plates (10 plates with 1x Dilution Buffer) or tubes (10 tubes of 10x Dilution Buffer). Both versions of the kit include a DNase I Treatment consisting of 1 tube of DNase I (RNase-Free) and 5 tubes of DNase Reaction Buffer. Both kits follow the same protocol and are designed to quantify multiple serotypes in various stages of the AAV production platform, i.e., from harvest to final purification, and can be used with standard PCR platforms.



Notes for storage:

- The DNase I should be stored at -25°C to -15°C.
- The DNase Reaction Buffer can be thawed and stored at room temperature.
- The Dilution Buffer can be thawed at room temperature but should be stored at 4°C when not in use. Avoid multiple freeze/thaw cycles.

Flip over to begin →

STEP 01

DNase I Treatment

Although AAV material may have been previously treated with endonucleases during the harvest stage of AAV production, applying DNase prior to performing PCR analysis is crucial to remove all exogenous DNA to promote accurate titer.

1. Thaw DNase I Treatment Reagents at room temperature, keep DNase I on ice.
2. In a cooling block or on ice, add the following reagents according to Table 1 into sterile PCR tubes. Mix gently by pipetting up and down 10 times. Briefly spin down to ensure all droplets are collected at the bottom of the tubes.
3. Incubate tubes at 37°C for 30 mins in a thermocycler. After incubation, spin down samples and place back on the cooling block or ice in preparation for dilution.

Table 1: DNase I Treatment

Reagent	Volume (µL)
DNase Reaction Buffer	40
DNase I	5
AAV Sample	5
TOTAL	50

Note: The volumes shown in Table 1 are for one single sample. If you are processing multiple samples, we recommend preparing and dispensing a master mix prior to adding your AAV sample.

STEP 02

Dilution and Capsid Lysis

OPTION 1: 1x PCR Dilution Plates

The 96-well plate format will contain frozen and sealed 96-well plates containing 90 µL of ready to use 1x Dilution Buffer.

1. Preheat a thermocycler to 95°C.
2. Thaw the required number of plates for ~20 mins until buffer reaches room temperature. Centrifuge the plate for a minimum of 2 minutes at 2500 x g to collect all liquid at the bottom of the wells.

TIP: Neglecting this step will lead to inaccurate dilutions and higher percent CV's.

3. Carefully add 10 µL of DNase treated AAV Sample to the first well of your dilution series. Mix well by pipetting up and down at least 15 times. Serially dilute by transferring 10 µl from the first well to the next well. Continue across the plate as needed until your sample(s) is in the range for your PCR reaction.
4. Centrifuge the plate briefly to draw all liquid to the bottom of the wells.
5. Place the plate in the pre-heated thermocycler at 95°C for 10mins, then rapidly cool down to 4°C at a rate of 3°C/s.
6. Samples are now ready for PCR. You can transfer your prepared DNA immediately into your master mix after the capsid disruption step is complete, or store for 24 hours at 4°C.

OPTION 2: 10x PCR Dilution Buffer

1. Preheat a thermocycler to 95°C.
2. Thaw the 10x Dilution Buffer at room temperature then dilute down to 1x prior to use. 1 tube of 10x solution can make 10mL of 1x Dilution Buffer. Thaw only the number of tubes you will need to avoid unnecessary freeze/thaw cycles.
3. Centrifuge briefly to collect all liquid to the bottom of the tube.
4. Dilute thawed 10x Dilution Buffer to 1x in PCR grade water and dispense into PCR tubes or plates depending on the number of samples and dilution range. Centrifuge briefly to collect all liquid to the bottom of the wells.
5. Place diluted samples in the pre-heated thermocycler at 95°C for 10mins, then rapidly cool down to 4°C at a rate of 3°C/s.
6. Samples are now ready for PCR. You can transfer your prepared DNA immediately into your master mix after the capsid disruption step is complete, or store for 24 hours at 4°C.

Note: This kit is only for DNA sample preparation and does not contain reagents for a PCR reaction.

→ Choose your method :

OPTION 1: 1x Dilution Buffer in pre aliquoted 96-well plates (90 µl per well) for your convenience when processing multiple samples.

OPTION 2: 10x Dilution Buffer in 2 mL tubes gives you the flexibility to prepare just the amount of reagent you need.

TIP: Both the 10x and 1x Dilution Buffers can be thawed at room temperature. However, they are sensitive to multiple freeze/thaw cycles.