

AAVPrime™ AAV-qPCR 滴度检测试剂盒

Cat. Nos. AA301 / AA302

产品概述

AAVPrime™ AAV-qPCR滴度检测试剂盒采用TaqMan探针法，可用于检测各种不同的血清型（只要你所构建的AAV载体的ITRs同样来源于AAV2），可高效、快速、准确地测定病毒拷贝数。该试剂盒主要包括DNA酶I、AAV裂解液、TaqMan-qPCR检测试剂、探针试剂以及标准品对照。

产品优势

- 能够判断AAV病毒是否包装成功
- 准确测定病毒拷贝数，以确保细胞转导和基因表达的效率
- 提供滴度检测过程从病毒的DNA提取到qPCR检测所需要的试剂
- 基于AAV病毒ITRS(末端反向重复序列)的定量检测
- 能够检测所有的血清型（只要你所构建的AAV载体的ITRs同样来源于AAV2）
- 简便的AAV基因提取流程，快速、准确得出测定结果

试剂盒组分

AA301 (20次DNA酶反应, 50次qPCR反应); A302 (40次DNA酶反应, 100次qPCR反应)。

Component	Volume	Concentration	Shipping	Storage
DNase I (RNase-free)	1x20 µl 2x20 µl	2,000 units/ml	Ice pack	-20°C, Stable for at least 12 months
DNase I buffer (10x)	1x20 µl 2x20 µl		Ice pack	-20°C, Stable for at least 12 months
AAV Lysis buffer	1x200 µl 2x200 µl		Ice pack	-20°C, Stable for at least 12 months
AAV-TaqMan qPCR mix (2x)	1x500 µl 2x500 µl		Ice pack	-20°C, Stable for at least 12 months
AAV-qPCR primer mix	1x100 µl 2x100 µl	2.5 µM	Ice pack	-20°C, Stable for at least 12 months
AAV-Probe	1x100 µl 2x100 µl	2.5 µM	Ice pack	-20°C, Stable for at least 12 months
AAV-qPCR standard (DNA)	1x25 µl 2x25 µl	1x10 ⁹ copies/µl	Ice pack	-20°C, Stable for at least 12 months
ddH ₂ O	1x1 ml 2x1 ml		Ice pack	Room temperature, Stable for at least 12 months

其他所需实验材料

用于细胞培养的设备

Real-time PCR仪器和耗材

相关产品

HEK293T AAV包装细胞系

转染试剂 (e.g., Endofectin-Lenti 转染试剂, GeneCopoeia cat.#EF001)

实验方法

实验前请仔细阅读整个实验步骤说明。本实验说明将从收获AAV病毒开始。

1. 收获AAV病毒

- 1) 准备一个干冰-乙醇浴和一个37°C 水浴。
- 2) 用细胞刮刮下AAV包装细胞（在10 cm 皿中转染AAV包装质粒 72 h），将细胞和上清收集到15 mL离心管中。
- 3) 将 2) 中收集到的悬液在干冰-乙醇浴和37°C 水浴间反复冻融4次，每次解冻后需要斡旋片刻。
- 4) 10000 x g 室温离心10 min去除细胞碎片。
- 5) 将上清转移至新管中，这就得到粗病毒。
- 6) 粗病毒可直接用于滴度检测。对于纯化后的以及浓缩的AAV病毒，为了让检测结果落在标准曲线范围内，则需要进行梯度稀释后才能进行滴度检测。
- 7) 粗病毒和纯化的病毒可长期保存于-80°C。

2. AAV基因组的提取

1) 用 DNase I 处理AAV病毒颗粒（粗病毒/纯化的病毒），去除游离细胞基因组及包装质粒。

DNase I 反应。用 200uL 管子进行以下反应 (总体积 10 μ L):

粗病毒 / 稀释后纯化的病毒*	8 μ L
10x DNase I Buffer	1 μ L
DNase I	1 μ L
Total	10 μ L

孵育:

①37°C , 20 分钟 (去除游离基因组DNA和质粒)

②95°C , 10 分钟 (使 DNase I失活)

③8°C, ∞

(如果用热循环仪进行孵育, 第①步时不要启动热盖功能)

*纯化的AAV需要用PBS进行稀释。稀释倍数由粗病毒的浓度决定。

2) AAV病毒颗粒的裂解

在 1) , 中加入 10 μ L AAV Lysis buffer, 涡旋后瞬离, 然后进行孵育:

①65°C , 30 分钟

②95°C , 10 分钟

③8°C, ∞

(如果用热循环仪进行孵育, 第①步时不要启动热盖功能)

该裂解样品将用于下一步的Real-time qPCR反应。

为确保 AAV 测定样品落在标准曲线的线性范围之内, 请将 2-2) 进行以下梯度稀释后取样:

A: Original lysate without dilution	8 µL
B: 10x dilute	8 µL
C: 100x dilute	8 µL
D: 1000x dilute	8 µL

3) Real-time qPCR反应

1) 准备制作标准曲线样品

稀释阳性标准品制作标准曲线。(每个稀释梯度取 5 µL 作为模板进行 qPCR 反应)

(1) 2×10^7 copies/µL (5 µL of AAV-qPCR standard (DNA) + 245 µL of ddH₂O)

(2) 2×10^6 copies/µL (5 µL of (1) + 45 µL of ddH₂O)

(3) 2×10^5 copies/µL (5 µL of (2) + 45 µL of ddH₂O)

(4) 2×10^4 copies/µL (5 µL of (3) + 45 µL of ddH₂O)

(5) 2×10^3 copies/µL (5 µL of (4) + 45 µL of ddH₂O)

(6) 2×10^2 copies/µL (5 µL of (5) + 45 µL of ddH₂O)

2) qPCR 反应体系 (总体积 20 µL):

AAV-TaqMan qPCR mix (2x)	10 µL
DNA standard or lysate sample	5 µL
Primer Mix (final concentration 0.25 µM each)	2 µL
Probe (final concentration 0.25 µM)	2 µL
ddH ₂ O	up to 20 µL
Total	20 µL

Notes:

- 将反应体系中的各个组分进行预混 (除了 DNA 标准品和样品) 后再进行分管。
- qPCR 反应中要设置无模板(NTC) 组。
- 标准品取样:

DNA 标准品	1×10^8	1×10^7	1×10^6	1×10^5	1×10^4	1×10^3
从 1) 中取样	5 µL of (1)	5 µL of (2)	5 µL of (3)	5 µL of (4)	5 µL of (5)	5 µL of (6)

3) qPCR 反应程序

以下反应程序适用于 ABI-ViiA 7 real time PCR 检测系统。您可能需要根据您所使用的检测系统进行微调您的反应程序。

Cycle	Steps	Temperature	Duration	Read
1	Denaturation	95 °C	10 min	off
35	Denaturation	95°C	10 sec	off
	Annealing	60 °C	20 sec	off
	Extension	72 °C	25 sec	on

荧光检测: FAM

淬灭: BHQ1

4) 数据分析

(1) 读取每一个标准品的 Ct 值, 以 LOG (拷贝数) 和 Ct 值生成标准曲线。标准曲线的相关系数应高于 0.99。

(2) 读取样品的 Ct 值, 代入(1)中生成的公式, 计算其对应的拷贝数。

(3) 将上述拷贝数乘以稀释系数, 得到原始样本的拷贝数(copies/ml)。

$$\text{稀释系数} = \frac{\text{DNase reaction volume}(\mu\text{l})}{\text{Original sample volume}(\mu\text{l})} \times \frac{\text{Lysis reaction volume}(\mu\text{l})}{\text{Volume of sample use in lysis reaction}(\mu\text{l})} \times \frac{1000\mu\text{l/ml}}{\text{Volume added to PCR well}(\mu\text{l})}$$

注:

- DNase reaction volume: 10 μL (根据本实验流程)
- Original sample volume: The volume of AAV particles used for DNase reaction. 8 μL (根据本实验流程)
- Lysis reaction volume: 20 μL (根据本实验流程)
- Volume of sample use in lysis reaction: 10 μL (根据本实验流程)
- Volume added to PCR well: 5 μL (根据本实验流程)

(4) 每一个 AAV 病毒颗粒含有单拷贝的单股正链的 DNA 基因组, 因此, 得到的拷贝数与病毒颗粒数一一对应。

(5) 标准曲线的例子：

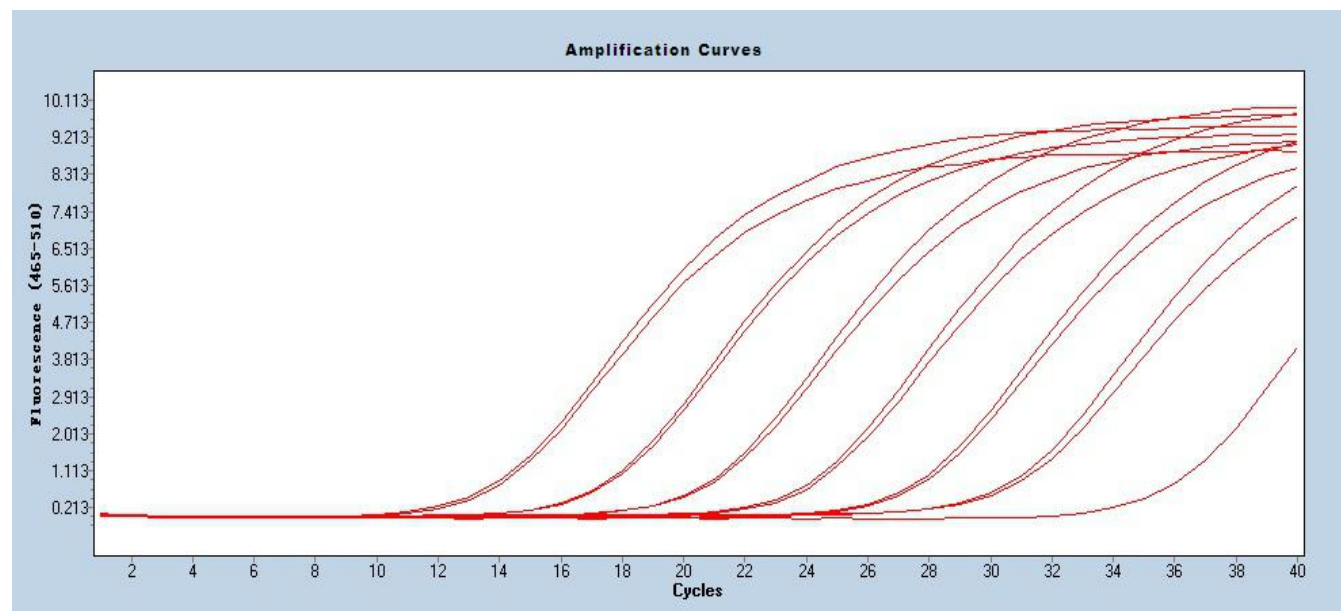


Figure 1. AAV-qPCR 标准品 (DNA) 和无模板对照(NTC)的扩增反应

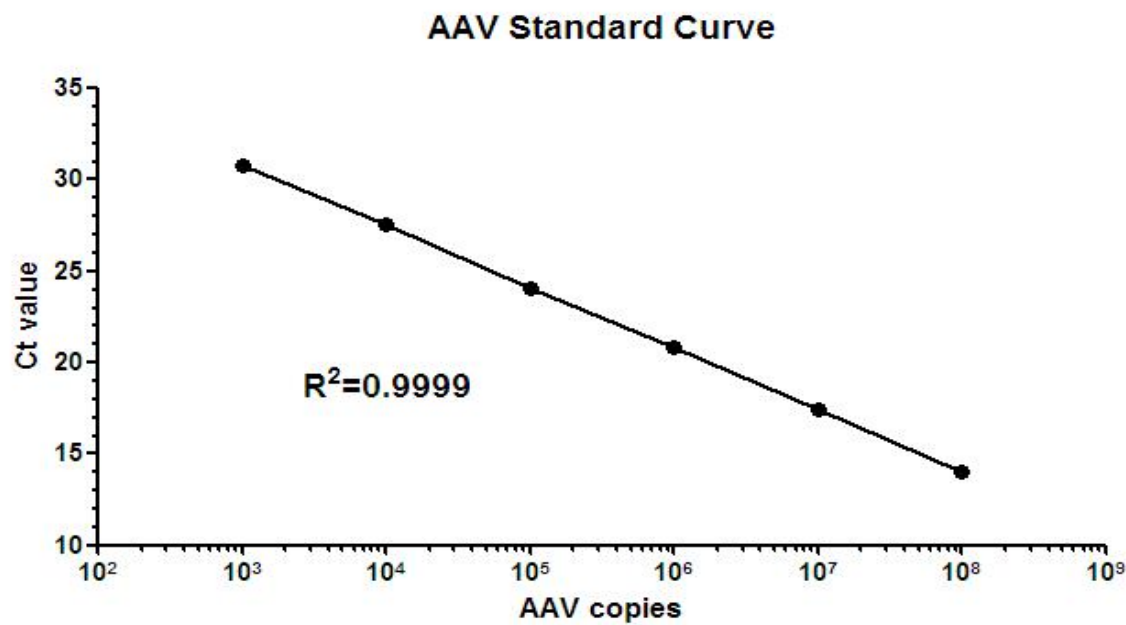


Figure 2. AAV-qPCR 标准品(DNA)制作的标准曲线。由 6 个 10 倍稀释梯度（ 1×10^3 到 1×10^8 拷贝）的标准品生成。

Limited Use License and Warranty

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Limited warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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