AAV8 ELISA Kit

Catalog Number: A80111A-96

【INTENDED USE】

The AAV8 titer detection kit independently developed by Junyan Biological can accurately, specifically and conveniently detect AAV8 virus particles in cell culture supernatants and purified virus preparations, and can be used for the determination of complete AAV8 wild-type virions, AAV8 recombinant virions and fully assembled AAV8 empty capsids.

【PRINCIPLE】

This kit uses double antibody sandwich enzyme-linked immunosorbent assay to determine the titer of AAV8 intact capsids in samples. The captured antibody was coated with 96-well enzyme label plate to prepare solid phase antibody, and then the standard and test samples were added, biotin-labeled detection antibody was added, and horseradish peroxidase (HRP) labeled streptavidin Strep -HRP was added to form a solid phase antibody-AAV8-detection antibody-Strep-HRP complex. After the reaction was finished, the TMB substrate was washed and then added for color reaction. The substrate changed from colorless to blue under the catalysis of HRP, and changed into the final yellow under the action of the termination solution (as shown below). The absorbance value (OD value) was measured at a specific wavelength, and a standard curve was established based on the titer value and absorbance value of the standard product. The absorbance value of the sample was substituted into the standard curve (it is recommended to use a four-parameter Logistic mathematical model to fit the equation) to calculate the complete capsid titer of AAV8 in the sample to be measured.

【 PRODUCT PERFORMANCE INDICATORS】

Detection limit: ≤2.00E+08 capsid/mL

Quantification limit: 4.13E+08 capsid/mL

Linear range: 1.32E+10-4.13E+08 capsid/mL

Accuracy (reciprocal recovery rate): 80%-120%

Accuracy (measurement deviation): ≤|±15%|

Repeatability (batch difference): ≤10%

【MATERIALS PROVIDED】

kit	Component	Size	Storage
Box 1	Microtiter Plate	96 T	2-8℃
	Biotin-Conjugate Antibody (20×)	750 μL×1 tube	2-8℃ (keep in dark)
	Strep-HRP (20×)	750 μL×1 tube	2-8℃ (keep in dark)

	Sample Dilution	60 mL×l vial	2-8℃
	Biotin-Conjugate Antibody Dilution	15 mL×l vial	2-8℃
	Strep-HRP Dilution	15 mL×l vial	2-8℃
	Wash Buffer (20×)	30 mL×l vial	2-8℃
	Developing Solution A	8 mL×l vial	2-8℃ (keep in dark)
	Developing Solution B	8 mL×l vial	2-8℃ (keep in dark)
	Stop Solution	15 mL×l vial	2-8℃
	Microplate Sealers	4	-
Box 2	Standard (1.32E+11 capsids/mL)	150 μL×4 tubes	Below -70℃

【STORAGE】

The standard product is stored below -70℃, and the remaining components are stored at 2-8℃, with a validity period of 12 months. Kits that have not been used after opening should be kept under the specified storage conditions. The production date and expiration date can be found on the kit label.

【REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED】

1. Enzyme marker, constant temperature oscillator (or constant temperature incubator), washing machine, vortex oscillator, timer;
2. High-precision pipettes and disposable suction heads (0.5-10 μL, 10-100 μL, 30-300 μL, 100-1000 μL);
3. Deionized water;
4. Absorbent paper, EP tube, disposable gloves.

【REAGENT PREPARATION】

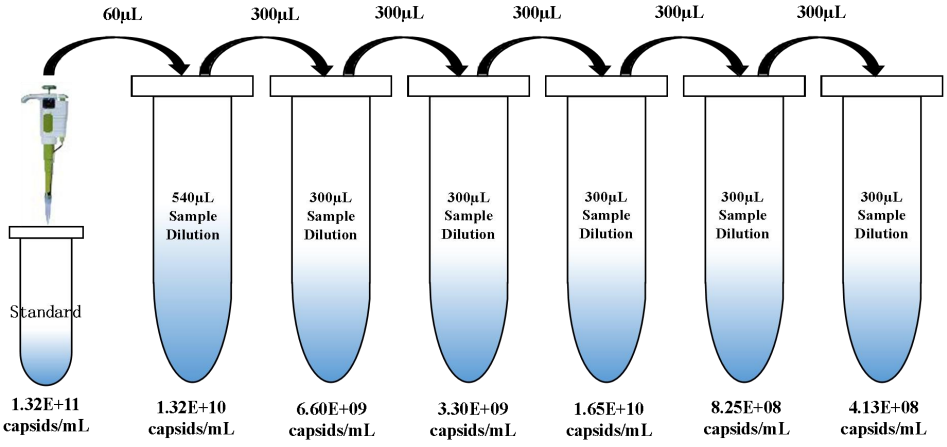
- Wash Buffer(1×):Take 1 part of Wash Buffer (20×) and add 19 parts of deionized water to prepare a working concentration wash buffer (1×). If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- Biotin-Conjugate Antibody (1×): Take detection antibody (20×) and dilute it with detection antibody diluent for reserve use. For example: Take the detection antibody (20×) 10μL and the detection antibody diluent 190μL and mix well.
- Strep-HRP (1×): Take Strep-HRP (20×) and dilute it with Strep-HRP diluent for reserve use. For example, take Strep-HRP (20×) 10μL plus Strep-HRP diluent 190μL and mix well.

- Preparation of color developing liquid: Mix the volume of color developing liquid A and B, and place away from light after mixing. (Note: Can not be placed for too long, generally used before 10min preparation. Do not use if the color solution has turned blue after mixing).

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃ .
2. Preparation of the standard curve: dilute the Standard with Sample dilution to 1.32E+10 capsids/mL, then prepared by Multiple Dilution method.



3. Sample incubation: Add the prepared standard and sample into the enzyme label plate (recommended addition sequence: standard hole, blank hole, sample hole, labeled sample hole, standard hole according to the titer gradient), 100 μL/ hole, seal the plate with sealing plate film, and then incubate at 37℃ for 30min.(Note: The sampling time should be controlled within 5min to avoid drift over time. If the plate is not sealed or the plate is not completely sealed during warming, the reaction liquid will evaporate, resulting in experimental errors.)
4. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5s can be slightly shaken after adding wash buffer).
5. Incubation for Biotin-Conjugate Antibody (1×): Add Biotin-Conjugate Antibody (1×), 100 μL/ well, seal the plate with sealing plate film, and then incubate at 37℃ for 30min. (Note: Before adding liquid after washing the board each time, check whether the slats are fixed to prevent liquid splashing caused by fixing the slats after adding liquid.)

6. Wash the board: same step (4)
7. Strep-HRP (1×) warming: add Strep-HRP (1×), 100 μL/ well, seal the board with sealing plate film, and then incubate at 37℃ for 30 min.
8. Wash the plate: same as step (4)
9. Color development: Add the pre-configured color development solution to the enzyme label plate, 100 μL/ hole, seal the plate with the sealing plate film, and incubate at 37℃ for 20 min away from light.
10. Termination: Add the termination liquid, 100 μL/ well, and the reading can be completed after uniform color development (Note: generally completed within 10min after adding the termination liquid).
11. Reading: Put the enzyme plate into the enzyme marker, set the wavelength to dual wavelength 450/630 nm, and read the absorbance value (Note: it is recommended to set the shock of 5-10 s in the reading program of the enzyme marker).

【CALCULATION OF RESULTS】

1. Calculation of optical absorption calibration value. The optical absorption calibration value of each standard or sample is: Absorbance value of standard product/sample hole (OD_{450nm} - OD_{630nm}) - absorbance value of blank control hole (OD_{450nm} -OD_{630nm}).
2. Take the titer of standard product (recommended that the logarithm of base 10 be used for standard titer X) as the horizontal coordinate (X), and the calibration value of light absorption of standard product as the vertical coordinate (Y), and draw the standard curve. Four-parameter Logistic mathematical model is recommended to fit the equation:

$$Y = ((A - D) / (1 + (X / C) ^ B)) + D$$

3. The light absorption calibration value [standard/sample hole absorbance value (OD_{450nm} - OD_{630nm}) - blank control hole absorbance value (OD_{450nm} -OD_{630nm})] is substituted into the formula to calculate the AAV8 titer in the sample.
4. If the OD value of the sample to be measured exceeds the OD value of the highest point of the standard curve, the sample needs to be diluted and re-determined.

【PRECAUTIONS】

1. All components are thoroughly mixed before use to ensure the uniformity of the reagent, and the mixed reagent needs to be centrifuged for a short time so that the liquid on the tube wall and lid is concentrated at the bottom of the tube.
2. The kit must be used within the validity period, and the corresponding standard curve must be prepared for each test. It is not recommended to use different batches of related reagents in mixed batches.

3. Do not touch the bottom of the microplate when adding liquid to the enzyme label plate to prevent damage to the coating layer. Change the feeding tank and suction head in time between different samples and steps to avoid cross-contamination.
4. When the slats are washed and patted dry, pay attention to prevent the slats from falling off, and the sealing plate film cannot be reused.
5. High titer may produce black flocculent during color development, which is a normal phenomenon, and the degree of slight does not affect the final reading result.
6. When reading, pay attention to check whether the detection wavelength and fitting equation are selected correctly.
7. Only by strictly following the operating methods of the instructions, all the reagents of this kit can be used to ensure the best detection effect.
8. The difference in test results can be caused by a variety of factors, including the effectiveness of the reagent, the operator's operation method, the storage of the kit and the test environment are closely related.
9. The company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible use of the sample before use, and reserve sufficient samples.
10. This kit is for research use only and is not intended for clinical diagnosis.

【SAFETY INSTRUCTIONS】

1. All biological samples have potential biosafety risks, and users should handle and discard samples in strict accordance with local laws and relevant regulations.
2. For safety reasons, the operator should wear personal protective equipment, such as lab coats, gloves, masks and goggles.

【PROBLEM ANALYSIS】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color	The incubation time is too short	Guarantee sufficient incubation time

development is weak or colorless	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly

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