Version 28-10/25

User's Manual

ResDetFast™ rcAAV-2/N Assay Kit

REF **DDNAF-008**

Σ **100 Reactions**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is intended for quantitative detection of the contamination rate of rcAAV-2/N* in serotype rAAV-2/N (where N represents different capsid serotypes). Sample types include, but are not limited to, recombinant adeno-associated virus vector harvest fluid, production end-stage cells, purified viral vector stock, and cell samples for detecting rcAAV based on cell culture methods.

***Note:** Before using this kit, please ensure the following:

1) AAV serotype;

2) The Inverted Terminal Repeat (ITR) of the test sample rAAV matches the following sequence:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCC
CGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATC
ACTAGGGGTTCCT

Principles of Testing

This kit utilizes the probe-based qPCR and can qualitatively detect the contamination rate of rcAAV in rcAAV-2/N (where N represents different capsid serotypes). The kit contains two systems: the Target system for detecting rcAAV and the Reference system for detecting rAAV; both systems' calibrators are included in the same quantitative reference, enabling rapid, sensitive, and specific quantitative detection of both rcAAV and rAAV simultaneously.The kit includes an internal control (IC), which can be added during the sample extraction phase to assess the extraction efficiency or be added during the PCR amplification reaction phase to monitor for inhibition in the amplification reaction, preventing the occurrence of false-negative results. It also contains UDG enzyme to prevent contamination from amplified products. The kit features easy operation, rapid detection, strong specificity, and high sensitivity. The kit is designed to be used in conjunction with Creative Diagnostics's nucleic acid extraction or purification kits (Contact CD for Cat. No.).

Reagents And Materials Provided

1. Target qPCR MIX: 1mL × 2
2. Reference qPCR MIX: 1mL × 2
3. rcAAV-2/N Internal Standard: 1mL × 1
4. rcAAV-2/N Negative Control: 1mL × 2
5. rcAAV-2/N Standards (ST1 to ST5): 1mL each, 2×10^1copies/μL, 2×10^2copies/μL, 2×10^3copies/μL, 2×10^4 copies/μL and 2×10^5copies/μL

Note: Components from different batch of kits should not be interchanged.

**If the internal standard function of the kit is not used, do not add the internal standard during extraction, do not set the ROX channel during program setup, and do not pay attention to the ROX results during result analysis.*

**If the sample does not contain the internal standard during extraction but is added during amplification, add 1 μL Internal Standard to each reaction when preparing the MIX, and dispense 21μL/reaction when aliquoting the qPCR MIX, resulting in a total PCR reaction volume of 41μL/reaction.*

Materials Required But Not Supplied

1. Real-time PCR instrumentation

   Supported models (including, but not limited to): ABI 7500, ABI QuantStudio™ 5, Roche LightCycler 480, SLAN-96P/SLAN-96S, and Bio-Rad CFX96 Real-Time PCR System
2. 96-Well qPCR Plate
3. Sterile low adsorption pipet tips (Nuclease-free, DNA-free, Aerosol-resistant pipet tips) (10μL, 100μL, 1000μL)
4. Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free) to prepare working solution, dilutions, and mixes (1.5mL)

Storage

Store at ≤-20°C in the dark, with a shelf life of 24 months.

Avoid repeated freezing and thawing, not exceeding 10 times.

Expiry date is indicated on the label.

Specimen Collection And Preparation

Pretreament: Before testing, the test samples must undergo nuclease treatment to eliminate interference from nucleic acid fragments that are not protected by protein capsids.

Instructions for Different Test Samples

This kit has different usage methods for direct qPCR and cell culture-based methods:

1. Direct qPCR method

1) Depending on the gene to be detected, the sample purification liquid needs to be diluted to varying degrees.
2) For Target gene detection, there is no need to dilute the sample purification liquid; For Reference gene detection, the sample purification liquid should first be diluted within the linear range (20 - 2.0×10^6 copies/μL).

   For example: If the concentration of the rAAV viral stock is known to be approximately 2.0E+12 VG/mL, then the sample purification liquid can be used directly for Target gene detection, but it should be diluted by at least 1000 times before being used for Reference gene detection.

2. Cell culture-based method:

1) Before cell transfection, determine the concentration of rAAV using the Reference qPCR MIX;

   Note: As mentioned above, the sample purification liquid to be tested also needs to be diluted within the linear range (20 - 2.0×10^6 copies/μL);
2) After cell transfection, determine the concentration of rcAAV using the Target qPCR MIX.

Sample Addition

Refer to the datsheet of Creative Diagnostics's Residual Nucleic Acid Extraction Kits for the operating steps. The internal standard sample volume is 10 μL and the sample volume is 100 μL.

Plate Preparation

The plate layout can refer to the table below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC-Target	NTC-Target	NTC-Target				ST1-Target	ST1-Target	ST1-Target	ST1-Reference	ST1-Reference	ST1-Reference
B	NCS-Target	NCS-Target	NCS-Target				ST2-Target	ST2-Target	ST2-Target	ST2-Reference	ST2-Reference	ST2-Reference
C	S-Target	S-Target	S-Target				ST3-Target	ST3-Target	ST3-Target	ST3-Reference	ST3-Reference	ST3-Reference
D	ERC-Target	ERC-Target	ERC-Target				ST4-Target	ST4-Target	ST4-Target	ST4-Reference	ST4-Reference	ST4-Reference
E	NTC-Reference	NTC-Reference	NTC-Reference				ST5-Target	ST5-Target	ST5-Target	ST5-Reference	ST5-Reference	ST5-Reference
F	NCS-Reference	NCS-Reference	NCS-Reference									
G	1/X S-Reference	1/X S-Reference	1/X S-Reference									
H	ERC-Reference	ERC-Reference	ERC-Reference									

**This example represents the detection of 1 no-template control (NTC), 1 negative control sample (NCS), 1 test sample, and 1 ERC, with 3 replicates for each test. In actual testing, the layout can be arranged according to the number of samples, referring to this example.*

**1/X indicates that the sample has been diluted X times*

Reagent Preparation

Take out Target qPCR MIX, Reference qPCR MIX, rcAAV-2/N Internal Standard, rcAAV-2/N Negative Control, and rcAAV-2/N Standards ST1 to ST5 from the kit, let them thaw at room temperature, mix well, and briefly centrifuge before use.

1. Extraction/Recovery Control (ERC)

Set the rcAAV-2/N spiking concentration in ERC as needed (taking the preparation of ERC with 2×10^4copies as an example). The specific steps are as follows:

1) Add 100 µL test sample to low DNA binding 1.5 mL microfuge tubes and lable "ERC".
2) Add 10 µL rcAAV-2/N Calibration ST3 to ERC tube, then vortex gently.
3) ERC and the same batch of test samples are subjected to sample pre-treatment together to prepare a sample ERC purification solution.

2. qPCR MIX

1) Calculate the required number of reaction wells based on the number of test samples. Generally make three replicate wells.

   Reaction wells = (5 concentration gradient standards + 1 No Template Control (NTC) + 1 Negative Control (NCS) + test samples + ERC) ×3

2) Calculate the total amount of qPCR MIX required (including the loss of 2 wells).

   qPCR MIX= (number of reaction wells + 2) × 20 µL

3) Place all reagents on ice, shake slightly and mix well. Refer to the following tables for sample addition.

Target System:

Standard	20 μL Target qPCR MIX + 20 μL ST1/ST2/ST3/ST4/ST5
NTC	20 μL Target qPCR MIX + 20 μL Negative Control
NCS	20 μL Target qPCR MIX + 20 μL NCS
Samples	20 μL Target qPCR MIX + 20 μL Samples
ERC	20 μL Target qPCR MIX + 20 μL ERC

Reference System:

Standard	20 μL Reference qPCR MIX + 20 μL ST1/ST2/ST3/ST4/ST5
NTC	20 μL Reference qPCR MIX + 20 μL Negative Control
NCS	20 μL Reference qPCR MIX + 20 μL NCS
Samples	20 μL Reference qPCR MIX + 20 μL Samples
ERC	20 μL Reference qPCR MIX + 20 μL ERC

qPCR Program Settings

Select FAM and ROX as fluorescence channels, where FAM is Target and Reference DNA and ROX is internal control. If the instrument is ABI series, select none for the reference fluorescence.

Set up the qPCR reaction program according to following Table.

Step	Temperature	Cycles	Time	Signal Collection
UDG	50℃	1×	2 mins	No
Initial Denaturation	95℃	1×	3 mins	No
Denaturation	95℃	45×	10s	No
Extension	60℃		30s	Yes

Analyze the results

1. Threshold Line Setting: The threshold line should be adjusted according to the noise level of the instrument, setting the threshold line above the fluctuation of the baseline signal. The threshold line setting should be based on laboratory verification data, with the detection limit requirements as the benchmark.
2. Interpretation of the Internal Standard: For negative results, the Ct value of the internal standard should be ≤35; for positive results, competition inhibition may occur, resulting in no value or poor performance of the internal standard.
3. Conditions for the Validity of the Test: The FAM channel of NTC and NCS displays no Ct value or Ct value≥35. The linear correlation of the standard curve should be $R^2 > 0.98$.
4. Result Analysis

Taking the SLAN-96P as an example,

1) If the threshold line needs to be adjusted, set the threshold to an appropriate value in the "Parameter

Settings" of the "Experiment Analysis" panel, with the analysis type set to quantitative.

2) In the "Plate Layout" panel, set the "Sample Type" column for the calibrators to "Standard," and assign the "Property" column with the values 2×10^1, 2×10^2, 2×10^3, 2×10^4 and 2×10^5 respectively (indicating the DNA concentration per well, in copies/μL), and name the corresponding "Sample Name" columns as ST1, ST2, ST3, ST4, ST5.
3) In the "Standard Curve" panel of "Experiment Analysis," you can read the slope, intercept, correlation coefficient, and amplification efficiency of the standard curve.
4) In the "Reaction Well Information Table" panel of "Experiment Analysis," the "Concentration" column can display the concentration value of the detection result, in copies/μL. Calculate the recovery rate of the sample and sample ERC based on the detection results, with the recovery rate required to be between 50% and 150%.

Taking the ABI 7500 qPCR instrument and software version 2.4 as an example,

1) If the threshold line needs to be adjusted, in the "Amplification Plot" panel of "Analysis," set the Threshold for the FAM and ROX channels to an appropriate height and click "Analyze."
2) In the "Plate Setup" panel of "Setup," set the "Task" column for the standard curve wells to "Standard," and assign the "Quantity" column with the values 2×10^1, 2×10^2, 2×10^3, 2×10^4 and 2×10^5 respectively (indicating the DNA concentration per well, in copies/μL). Name the corresponding "Sample" columns as ST1, ST2, ST3, ST4, ST5. Set the "Task" column for the no-template control (NTC) well to "NTC," and set the "Task" column for the negative control (NCS) well, the test sample well, and the sample ERC well to "Unknown," and name the corresponding "Sample Name" columns as NCS-Reference, NCS-Target, 1/x S-Reference, S-Target, ERC-Reference, ERC-Target.
3) In the "Standard Curve" panel of "Analysis," you can read the Slope, Y-Intercept, R^2, etc., of the standard curve.
4) In the "View Well Table" panel of "Analysis," the "Quantity" column can display the concentration value of the detection result, in copies/μL. Calculate the recovery rate of the sample and sample ERC based on the detection results, with the recovery rate required to be between 50% and 150%.

   Calculate the contamination rate of rcAAV in rAAV using the following formula:

   Contamination Rate = Target Detection Value ÷ (Reference Detection Value × Dilution Factor ÷ 2)

Note: Each rAAV contains two reference genes.

The parameter settings for the example result analysis provided above are for reference only. Specific settings should be based on the laboratory equipment model and the software version used, and generally can also be automatically interpreted by the instrument.

Precision

LOQ	CV≤20%
Medium to High Values	CV≤15%

Specificity

ResDetFast™ rcAAV-2/N Assay Kit has no cross-reactivity with HEK293T, Sf9, Hi5, CHO, E.coli, Pichia

pastoris, VERO, E.coli, Staphylococcus aureus, Staphylococcus epidermidis, mycobacterium, etc., and has no interference.

Recovery

50-150%

Precautions

1. The kit should be stored and transported at or below -20°C.
2. Before the experiment, please read the instructions of this kit carefully and strictly follow the operating steps. Precise control of time, reagent volume, etc., during the operation process can yield the best results.
3. Ensure that consumables related to nucleic acid extraction are clean, free from DNase/RNase, and free from HEK293 nucleic acid and related products. The extraction process should be as low-temperature and rapid as possible, and after completion, proceed to the next experiment or freeze for preservation.
4. Do not use expired components or mix components from different batches.
5. Thaw reagents completely at room temperature before use, and briefly centrifuge to ensure the liquid settles at the bottom of the tube. Avoid repeated freezing and thawing to prevent affecting the performance of the reagents.
6. For top-illuminated instruments, use new disposable PE gloves to seal the reaction tubes. For bottom-illuminated instruments, avoid touching the bottom of the reaction tubes with bare hands or used gloves. Use disposable latex gloves without fluorescent substances during the detection process.
7. The laboratory should be strictly managed according to the relevant regulations, and genetic testing should be carried out in the order of reagent preparation area → template extraction area → amplification area → analysis area. There should be strict requirements for personnel, equipment, reagents, and air flow between each area.
8. Samples, calibrators, and other materials should be promptly capped after use to avoid cross-contamination and aerosol pollution that may cause false positives.
9. Amplified products are prohibited from being opened, and waste generated during the experiment should be collected in a timely manner and treated harmlessly away from the PCR laboratory.
10. If the target in the sample is strongly positive, the competitive inhibition of the system may affect the detection of the internal standard.
11. It is recommended to use the latest version of the software for each instrument for experiments and data analysis.