C001-EN.01

resDetect™ Cas9(CRISPR Associated Protein 9) ELISA Kit

(Residue Testing)

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：CAS-C001

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

resDetect™ Cas9(CRISPR Associated Protein 9) ELISA Kit (Residue Testing) is developed for quantitative detection of Cas9 in cell Therapy. It is intended for research use only (RUO).

BACKGROUND

CRISPR-Cas9 is the third generation of gene editing technology after ZFN and TALENs, which has the advantages of high editing efficiency, low cost, and easy operation, and has become the most mainstream gene editing method today. At present, several cell and gene therapy drugs based on CRISPR-Cas9 technology have been approved for clinical trials. In in vitro gene therapy, cells modified by the CRISPR-Cas9 system are tested for extracellular or intracellular Cas9 nuclease residues before being infused back into the body.

To support the development of CAR-T drugs, ACROBiosystems independently developed Cas9 ELISA kit via rigorous methodological validation, which is used for detection of GMP Cas9 in samples from CAR-T product preparation processing for evaluation the quality of CAR-T products in drug development and CMC quality control stages.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of Cas9 by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Cas9 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Cas9 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of Cas9 bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant samples.
3. Do not use reagents past their expiration date.

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4. Do not mix or substitute reagents with those from other kits or other lot number kits.

5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CAS01-C01	Pre-coated Anti-Cas9 Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
CAS01-C02	Cas9 Nuclease Standard	20 μg	Power	2-8°C	-70°C
CAS01-C03	Biotin-Anti-Cas9 Antibody	15 μg	Power	2-8°C	-70°C
CAS01-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CAS01-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
CAS01-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
CAS01-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CAS01-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

SRORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.

2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 5 µg.

Note: Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
CAS01-C02	Cas9 Nuclease Standard	20 µg	200 µg/mL	100 µL
CAS01-C03	Biotin-Anti-Cas9 Antibody	15 µg	100 µg/mL	150 µL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

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Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-Cas9 Antibody working fluid:

Dilute Biotin-Anti-Cas9 Antibody to 0.01μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

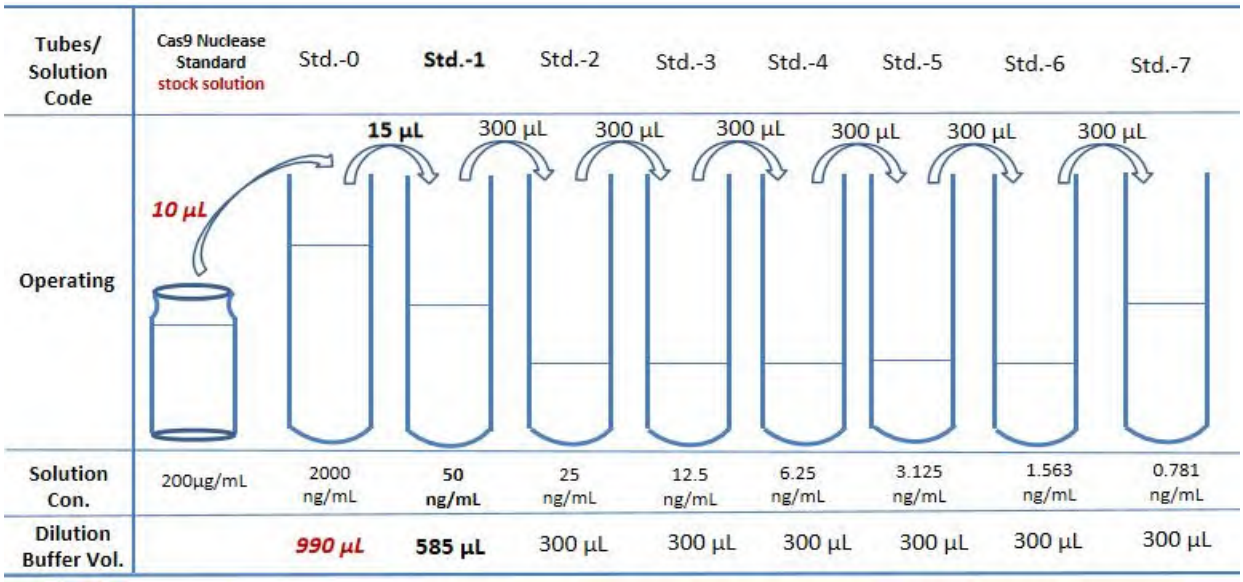
Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation

If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

2. Preparation of Standard curve

The concentration of the reconstituted Cas9 Nuclease Calibrator (CAS01-C02) is 200 μg/mL， prepare (Std.-0) by diluting 10 μL the reconstituted Cas9 Nuclease Calibrator into 990 μL Sample Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, **Std.-1 (50 ng/mL)**, by diluting 15 μL Std.-0 into 585 μL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

Add 100 μL Calibrator and samples to each well. For blank Control wells, please add 100 μL Dilution Buffer.

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***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at **37℃** for 1 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-Cas9 Antibody

For all wells, add 100 µL Biotin- Anti-Cas9 Antibody (dilute to 0.01µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at **37℃** for 1 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at **room temperature** for 1 hour.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at **room temperature** for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

***Note:** The color in the wells should change from blue to yellow.*

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14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

***Note**: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at $OD_{630\ nm}$.*

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 0.781-50 ng/mL. If the OD value of the sample to be tested is higher than 50 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.781 ng/mL, the sample should be reported.

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QUICK GUILD

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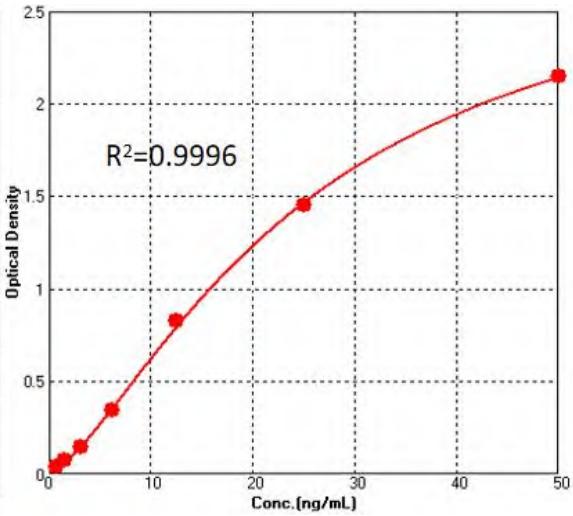
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
50	2.178	2.205	2.192	2.147
25	1.528	1.470	1.499	1.455
12.5	0.889	0.848	0.869	0.824
6.25	0.394	0.378	0.386	0.342
3.125	0.199	0.182	0.191	0.146
1.563	0.121	0.111	0.116	0.072
0.781	0.085	0.079	0.082	0.038
0	0.048	0.041	0.045	/



SENSITIVITY

The minimum detectable concentration of Cas9 is 0.347 ng/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision: Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision: Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (ng/mL)	38.419	11.111	1.844	39.098	10.358	1.792
SD	1.263	0.366	0.098	0.635	0.991	0.106
CV (%)	3.3%	3.3%	5.3%	1.6%	9.6%	5.9%

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***Note**: The example data is for reference only.*

RECOVERY

Three Cas9 with different concentrations were tested to calculate the recovery rate.

Sample(n=3)	Detect Conc.(ng/mL)	Average Detect Conc.(ng/mL)	Average Recovery (%)	Range (%)
High	37.034	36.176	90.4	88.2-92.6
	36.846			
	35.593			
	36.142			
	35.267			
Middle	9.977	10.050	100.5	94.6-106.6
	10.665			
	9.856			
	10.292			
	9.462			
Low	2.027	2.072	103.6	95.3-119.2
	2.385			
	2.016			
	2.027			
	1.906			

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of Cas9 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	102.6	93.6
	Range (%)	100.1-105.0	86.5-104.4
1:4	Average Recovery (%)	104.9	102.5
	Range (%)	101.0-109.8	101.9-102.9
1:8	Average Recovery (%)	102.5	107.4
	Range (%)	99.9-106.4	100.3-110.7
1:16	Average Recovery (%)	100.0	110.7

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	Range (%)	96.3-105.5	101.4-117.3

***Note**: The example data is for reference only.*

SPECIFICITY

This assay recognizes natural and recombinant Cas9. No cross-reactivity was observed when this kit was used to analyze the following recombinant factors.

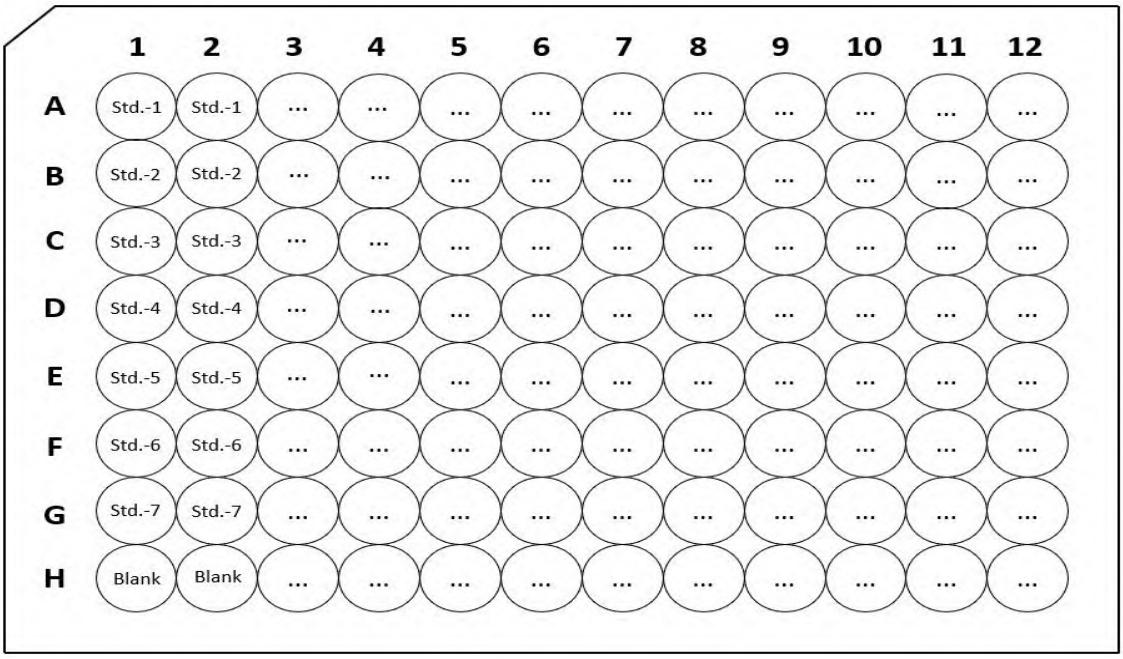
Reactivity	Cap-2'-O-Methyltransferase; T7 RNA Polymerase; Nuclease; Cas12a; Pyrophosphatase

INTERFERING SUBSTANCES

Verify potential matrix effects by adding different levels of Cell culture medium、DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
Cell culture medium (DMEM)	50%
Cell culture medium (1640)	50%
DMSO	1%
HSA	5%

PLATE LAYOUT



Note: Blank is a Blank Dilution Buffer hole.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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