



A054-EN.01

**resDetect™ Salt Active GENIUS™ Nuclease Nuclease ELISA Kit**  
**(Residue Testing)**  
**(Enzyme-Linked Immunosorbent Assay)**

**Pack Size: 96 tests**

**Catalog Number: RES-A054**

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

***For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures***

## **INTENDED USE**

The Salt Active GENIUS™ELISA Kit was developed for the detection and quantitative determination of nuclease in samples from downstream processing where nuclease is used as a process or purification aid. It is intended for research use only (RUO).

## **BACKGROUND**

Nucleases are enzymes that degrade nucleic acids, either DNA or RNA. It has been used for the preparation of nuclear extracts by digesting DNA and releasing nuclear proteins intimately associated with DNA. It has also been designed for removing RNA and DNA in biotechnological processing. Thus, the amount of residual nuclease in biological products should be detected and limited.

To support the development of biological products, ACROBiosystems has developed a self-developed universal nuclease quantitative assay kit for the detection and analysis of nuclease residues in biologics through rigorous methodological validation.

## **PRINCIPLE OF THE ASSAY**

This assay kit is used to measure the levels of Salt Active Nuclease by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Nuclease 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-Nuclease 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 Salt Active Nuclease 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.
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**

**Table 1. Materials provided**

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES054-C01	Pre-coated Anti-Nuclease Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES054-C02	Salt Active Nuclease Standard	5 µg	Power	2-8°C	-70°C
RES054-C03	Biotin-Anti-Nuclease Antibody	20 µg	Power	2-8°C	-70°C
RES054-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES054-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES054-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES054-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES054-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**

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

37°C Incubator;

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

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

## **REAGENT PREPARATION**

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37°C 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 vortexing. 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, the size of the aliquot (RES054-C02) should not be less than 2 µg, and the size of the aliquot (RES054-C03) 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.
RES054-C02	Salt Active Nuclease Standard	5 µg	50 µg/mL	100 µL
RES054-C03	Biotin-Anti-Nuclease Antibody	20 µg	200 µg/mL	100 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:

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

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

Dilute Biotin-Anti-Nuclease Antibody to 0.4 µg/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 Salt Active Nuclease Calibrator (RES054-C02) is 50 µg/mL, prepare (Std.-0) by diluting 10 µL the reconstituted Salt Active Nuclease Calibrator into 490 µL Sample Dilution Buffer, mix gently well. Then prepare Std.-1' by diluting 10 µL Std.-0 into 490 µL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (800 pg/mL), by diluting 24 µL Std.-1' into 576 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.

Tubes/ Solution Code	Salt Active Nuclease Standard <i>stock solution</i>	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Operating									
Solution Con.	50µg/mL	1000 ng/mL	20 ng/mL	800 pg/mL	400 pg/mL	200 pg/mL	100 pg/mL	50 pg/mL	25 pg/mL
Dilution Buffer Vol.		<b>490 µL</b>	<b>490 µL</b>	<b>576 µL</b>	300 µL	300 µL	300 µL	300 µL	300 µL

### 3. Add Samples

Add 100  $\mu$ L Calibrator and samples to each well. For blank Control wells, please add 100  $\mu$ L Dilution Buffer.

*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 room temperature for 1.0 hour.

### 5. Washing

Remove the remaining solution by aspiration, add 300  $\mu$ L of 1 $\times$ Washing Buffer to each well, soak for 10s, remove any remaining 1 $\times$ 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-Nuclease Antibody

For all wells, add 100  $\mu$ L Biotin- Anti-Nuclease Antibody (dilute to 0.4  $\mu$ g/mL) working solution. Please prepare it for one-time use only.

### 7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 8. Washing

Repeat step 5.

### 9. Add Streptavidin-HRP

For all wells, add 100  $\mu$ L 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 1.0 hour.

### 11. Washing

Repeat step 5.

### 12. Substrate Reaction

Add 100  $\mu$ L 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  $\mu$ L 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.*

#### **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  $OD_{630nm}$ .*

#### **CALCULATION OF RESULTS**

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).
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: 25 pg/mL-800 pg/mL. If the OD value of the sample to be tested is higher than 800 pg/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 25 pg/mL, the sample should be reported.

## QUICK GUID

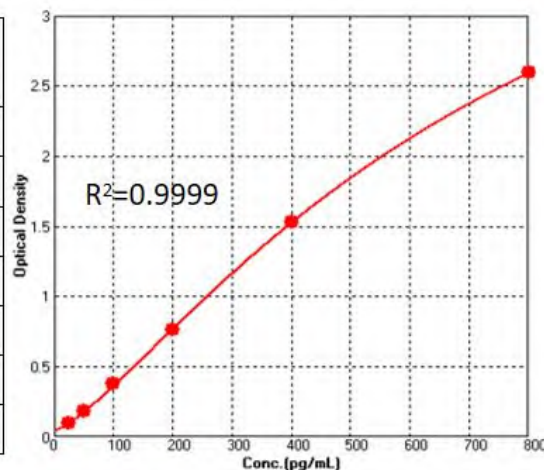




## TYPICAL DATA

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 (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
800	2.684	2.612	2.648	2.592
400	1.586	1.592	1.589	1.533
200	0.828	0.816	0.822	0.766
100	0.432	0.435	0.434	0.377
50	0.245	0.23	0.238	0.181
25	0.156	0.155	0.156	0.099
0	0.053	0.060	0.057	/



## SENSITIVITY

The minimum detectable concentration of Salt Active Nuclease is 9.330 pg/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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	595.045	292.052	58.987	590.865	289.715	60.114
SD	10.615	7.587	1.843	18.299	4.543	2.399
CV (%)	1.8	2.6	3.1	3.1	1.6	4.0

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

## RECOVERY

Three Salt Active Nuclease with different concentrations were tested to calculate the recovery rate.

Sample(n=3)	Detect Conc.(pg/mL)	Average Detect Conc.(pg/mL)	Average Recovery (%)	Range (%)
High	611.724	598.885	99.8	96.1-103.2
	599.007			
	619.420			
	576.751			
	587.523			
Middle	285.858	293.720	97.9	95.3-101.1
	289.731			
	291.923			
	297.813			
	303.273			
Low	66.062	67.596	112.7	110.1-117.9
	66.704			
	70.738			
	66.917			
	67.557			

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of Salt Active Nuclease 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 (%)	101.4	103.7
	Range (%)	96.0-108.2	98.5-112.4
1:4	Average Recovery (%)	97.6	101.7
	Range (%)	93.9-99.8	97.1-104.5
1:8	Average Recovery (%)	105.4	105.7
	Range (%)	102.3-108.7	101.8-109.3
1:16	Average Recovery (%)	113.9	107.6
	Range (%)	107.9-117.3	88.5-115.3

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

### **SPECIFICITY**

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

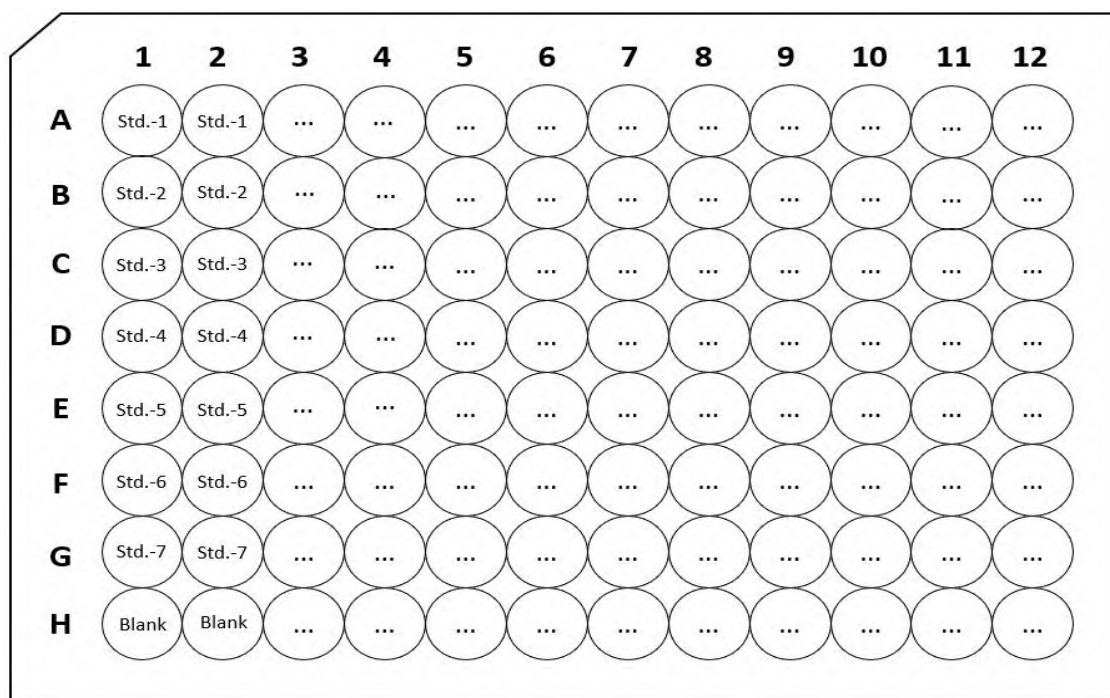
Reactivity	Cap-2'-O-Methyltransferase; T7 RNA Polymerase; CAS9; Cas12a; Pyrophosphatase
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### **INTERFERING SUBSTANCES**

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	5%
HSA	5%

### **PLATE LAYOUT**



*Note: Blank is a Blank Dilution Buffer hole.*

## TROUBLESHOOTING GUIDE

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash.
	Contaminated wash buffer	Make fresh wash buffer
Very low readings across the plate	Incorrect wavelengths	Check filters/reader
	Insufficient development time	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	Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay
	Reagents not at room temperature	Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts