

BenzoNuclease® ELISA Kit

Cat No.: PA018

01/ Product Description

BenzoNuclease® is a genetically engineered endonuclease from *Serratia marcescens*.

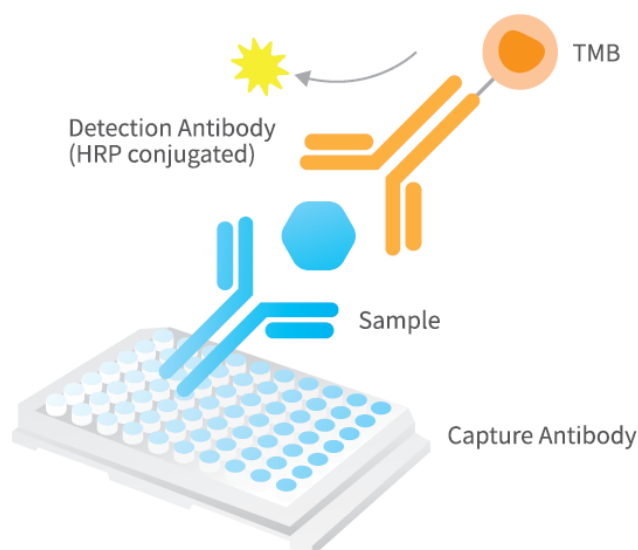
BenzoNuclease® acts as an endonuclease that degrades both DNA and RNA—whether single stranded, double-stranded, linear, circular or supercoiled. Upon complete digestion, all free nucleic acids present in solution are reduced to 5'-monophosphateterminated oligonucleotides, which are three to five bases in length.

BenzoNuclease® is an efficient enzyme for removing all forms of DNA and RNA from biological products. In processed biological products, there may be traces of BenzoNuclease® residues, and these traces of residues will have a certain impact on the application of subsequent biological products. The residual amount of BenzoNuclease® in biological products is one of the important indicators to measure the quality of biological products.

This kit can detect and quantitatively analyze the BenzoNuclease® residues in recombinant viral vectors and vaccine preparations with high sensitivity and specificity.

02/ Reaction Principle

This kit adopts the double-antibody sandwich ELISA method. The BenzoNuclease® capture antibody is pre-coated. After adding the sample, the sample is captured to form an antibody-antigen complex, and then the HRP-conjugated BenzoNuclease® detection antibody is added to form an antibody-antigen-antibody "sandwich" complex. Finally, TMB substrate was added for color development. After the reaction was terminated, the absorbance value (OD value) was read at a wavelength of 450 nm. The content of BenzoNuclease® in the sample was positively correlated with the OD value.



- Step 1: Test sample added to the well
- Step 2: Detection Antibody added to the well
- Step 3: Colorimetric detection with TMB substrate

03/ Temperature for Storage

When the kit is unopened, store it in a dry place at 2-8°C away from light, with a validity period of one year; after the kit is opened, use it as soon as possible.

04/ Product Packaging

Item	Description	Quantity
BenzoNuclease® Microplate	Coated capture antibody	96T/Kit
100× BenzoNuclease® Detection Antibody	HRP-conjugated, dilution ratio 1:100	150μl
BenzoNuclease® Standard	Recombinant BenzoNuclease®, 10μg/ml, dilution range 0.014-10.00ng/ml	150μl
20× Wash Buffer	-	25ml×2
1× Dilution Buffer 1	Sample dilution buffer	30ml×2
1× Dilution Buffer 2	Detection Antibody dilution buffer	15ml
Color Reagent A	-	6ml
Color Reagent B	-	6ml
Stop Reagent	-	12ml
Sealing Film	-	2 pieces

Note: Components of different batches cannot be mixed.

05/ Manual

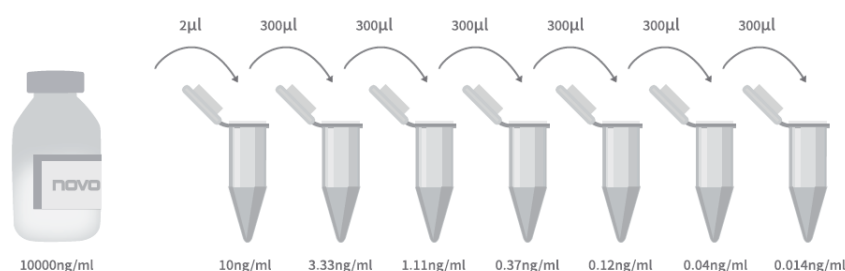
5.1 Preparation

All reagent components and samples to be tested need to be equilibrated to room temperature before use.

- Preparation of 1× Wash Buffer:** Equilibrate 20× Wash Buffer to room temperature until the crystals are completely dissolved. After mixing, dilute 20 times with deionized water or ultrapure water. The amount of 1× Wash Buffer per well is 3ml. It is stable for 1 week at 2-8°C.
- Preparation of 1× BenzoNuclease® Detection Antibody:** Mix well before use, then dilute 100× BenzoNuclease® Detection Antibody 1:100 with 1× Dilution Buffer 2.
- Preparation of Substrate Solution:** Mix Color Reagent A and Color Reagent B by 1:1, for immediate use, 100μl per well. Do not use if the mixture turns blue.
- BenzoNuclease® Standard dilution:** Dilute BenzoNuclease® Standard with 1× Dilution Buffer 1 to 8 concentrations of 10.00, 3.33, 1.11, 0.37, 0.12, 0.04, 0.014, and 0.00ng/ml. The specific dilution process is as follows:

Take 2μl BenzoNuclease® Standard and add 1998μl 1× Dilution Buffer 1 to prepare a 10ng/ml sample. After mixing, take 300μl of 10ng/ml sample and add 600μl of 1× Dilution Buffer 1 for serial dilution.

Note: A corresponding standard curve should be prepared for each test, and standard curves of different kits and different days cannot be mixed.

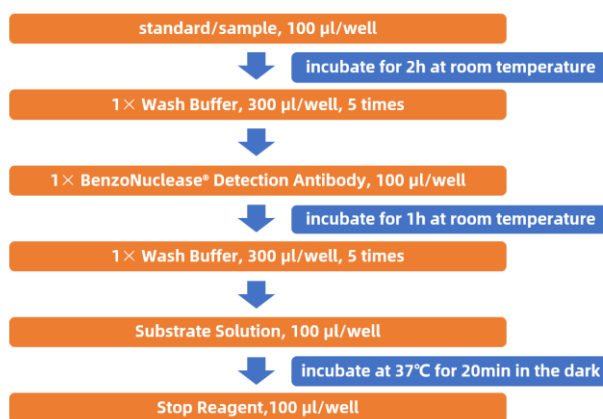


5.2 Test Procedure

All operations should be carried out at room temperature. It is recommended to test each sample no less than twice.

1. Sample incubation: Add the diluted BenzoNuclease[®] Standard and the sample to the BenzoNuclease[®] Microplate respectively, 100 μ l/well, mixed well, cover with a sealing film, and incubate at room temperature for 2h.
2. Wash plate: Discard the liquid in the well, wash the plate with 1 $\times$ Wash Buffer, 300 μ l/well, wash the plate 5 times, and pat dry.
3. BenzoNuclease[®] Detection Antibody incubation: Add the diluted 1 $\times$ BenzoNuclease[®] Detection Antibody to the BenzoNuclease[®] Microplate, 100 μ l/well, mix well, cover with sealing film, and incubate at room temperature for 1 h.
4. Wash plate: Repeat Step 2.
5. Color development: Add the prepared Substrate Solution to the BenzoNuclease[®] Microplate, 100 μ l/well, mix well, and incubate at 37°C for 20 min in the dark.
6. Stop the reaction: Add the prepared Stop Reagent to the BenzoNuclease[®] Microplate, 100 μ l/well, shake the plate gently until the color develops uniformly, and ensure that the color development time of each well is the same.
7. Measurement: Put the BenzoNuclease[®] Microplate into the microplate reader, it is recommended to read the absorbance by dual wavelength test (test wavelength 450nm, reference wavelength 630nm), and read the value within 20 minutes.

5.3 Concise Test Procedure



06/ Analysis

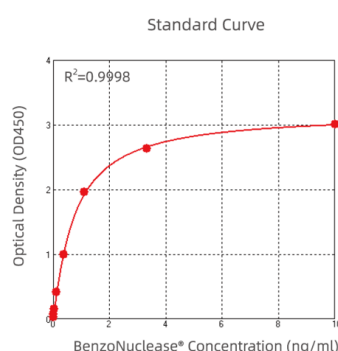
If the OD value of the sample to be tested exceeds the OD value at the highest point of the standard curve, the sample needs to be diluted and re-measured.

Draw a standard curve with the concentration of the standard as the abscissa and the light absorption value of the standard as the ordinate. It is recommended to use ELISACalc.exe regression/fitting calculation software for four-parameter fitting to calculate the concentration of BenzoNuclease[®] in the sample.

Example for reference:

The following standard curve diagram is for reference only, and the sample content should be calculated based on the standard curve drawn by the standard products in the same experiment.

ng/ml	OD450
10.00	3.01
3.33	2.63
1.11	1.96
0.37	1.00
0.12	0.41
0.04	0.15
0.014	0.07
0.00	0.03
R ²	0.99



07/ Product Performance Indicators

7.1 Sensitivity

The detection sensitivity of the kit was 0.014ng/ml.

7.2 Blank limit

The blank limit of the kit is ≤ 0.06 .

n=16 replicates	Value
AVE	0.0241
SD	0.0076
AVE+2SD ≤ 0.06	0.0393

7.3 Detection Range

The detection range of the kit is 0.014-10.00ng/ml.

7.4 Spike recovery rate

The spiked recovery rate of the kit was 80%-120%.

Theoretical concentration (ng/ml)	Back-calculated average concentration (ng/ml)	Average spike recovery
1.11	1.12	1.01
0.37	0.36	0.97
0.12	0.14	1.15

7.5 Precision

The intra-assay precision of the kit is $\leq 10\%$, and the inter-assay precision is $\leq 10\%$.

Theoretical concentration (ng/ml)	Intra-assay Precision		Inter-assay Precision	
	Back-calculated average concentration (ng/ml)	CV	Back-calculated average concentration (ng/ml)	CV
1.11	1.21	3.6%	1.18	5.5%
	1.15		1.09	
	1.13		1.24	
0.37	0.41	8.0%	0.36	7.7%
	0.39		0.41	
	0.35		0.36	
0.12	0.13	8.3%	0.12	7.7%
	0.12		0.13	
	0.11		0.14	

08/ Precautions

1. The reagents should be equilibrated to room temperature according to relevant requirements before the experiment.
2. Each reagent should be thoroughly mixed before use to ensure the uniformity and accuracy of the reagents.
3. Strictly control the reaction temperature and reaction time.
4. The measured value cannot exceed the normal range.
5. The times of washings, the amount of washing buffer, the soaking time of washing solution, and the strength of washing are all issues that should be paid attention to.
6. Try to avoid air bubbles in the microwells when terminating the reaction.
7. A corresponding standard curve should be prepared for each test. Standard curves from different kits and different days cannot be

mixed.

8. Pay attention to changing the sample tank and pipette tip in time between different samples and steps to avoid cross-contamination.
9. The reading of the light absorption value should be completed within 20 minutes after adding the stop solution.
10. High concentration may produce black flocs during color development, which is a normal phenomenon, and a slight degree will not affect the final reading result.

09/ Q&A

9.1 Common causes for high background problem:

1. Contaminated substrate solution: The substrate solution itself is colorless, ensuring that the substrate is not contaminated by metal ions or oxidizing reagents before use, and stored in the dark.
2. Incorrect dilution of the conjugate (BenzoNuclease® Detection Antibody): Make sure that the conjugate is diluted according to the specifications specified in the instruction manual. Too high conjugate concentration may cause high background.
3. Incorrect washes: Insufficient or missed washes can result in high background. Check the amount of wash buffer and make sure all wash steps are completed, let the wash buffer stay in the well for at least 15-30s, and make sure there is no residue after each wash.
4. Incorrect incubation time and temperature: The incubation time and temperature are strictly in accordance with the test procedure.
5. Contamination of consumables: Use clean tubes, pipette tips and other consumables.
6. Contaminated diluents: Most ELISA diluents are protein-based and are best prepared before use.
7. Sample contamination: Avoid sample cross-contamination.
8. The sample concentration is too high: determine the appropriate dilution ratio.

9.2 High CV value

1. Destroy the coating when washing/spitting: Be careful not to scrape the inside of the microplate when washing the plate or adding standards and samples.
2. Inhomogeneous sample: Mix the sample before adding to ensure that the sample is homogeneous.
3. Edge effect: Avoid incubating the plate under conditions with large temperature changes (such as vents or windows), and add the same volume of PBS to the outermost edge of the ELISA plate as the test sample. Do not stack the plates while incubating.
4. Incorrect washes: Insufficient or missed washes can lead to increased CV values. Do not reduce wash volume or wash steps, and allow wash buffer to sit in the microplate for 15-30 s.

9.3 Inaccurate standard curve

1. Standard dilution error: The concentrated standard was serially diluted and mixed correctly.
2. Standard storage issues: The standards and diluents should be stored in accordance with the instructions.
3. Measurement problem: There are air bubbles in the wells that cause abnormal readings. Try to avoid air bubbles in the micropores when terminating the reaction.

9.4 No signal or weak color developing

1. Reagent storage problem: The reagent has expired, the enzyme-conjugated antibody is inactivated or the titer is reduced, and different batches of diluents are used interchangeably.
2. The pH of the solution is incorrect: the pH of the general dilution should be maintained at 7.2-7.4.
3. Operation problems: Relevant reagents were added by mistake or missed, the reagents were not equilibrated to room temperature

before use, and the incubation time and incubation temperature did not meet the experimental requirements.

9.5 The measured value is out of the normal range

1. Dilution issues: Dilute the samples and standards according to the test procedure, using the diluent provided by the kit.
2. Measurement problems: Check the detection wavelength provided in the instruction manual to make sure the wavelength is correct.
3. Color development time: The time displayed in the operation process may need to be changed according to changes in temperature or other experimental conditions (temperature, shelf life of Kit, humidity). It is recommended to add stop solution when the highest concentration standard well appears dark blue.
4. Incubation issues: Incubation time and temperature are strictly in accordance with the test procedure, and the cover during incubation can prevent liquid evaporation and contamination.