NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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Nonspecific Endonuclease (SMNE) ELISA Kit User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1402421
Version: A/0
For Research Use Only

Biofargo, Inc.

■ Product Name

Nonspecific Endonuclease (SMNE) ELISA Kit.

■ Package

96 tests/Kit

■ Intended Use

This kit is suitable for the quantitation of Nonspecific Endonuclease (SMNE) in purification process of bioproducts. It can detect genetically engineered endonucleases from *Serratia marcescens*, such as Benzonase® endonuclease.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

■ Product Description

This kit is based on the solid-phase enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich technique to detect residual nonspecific endonuclease (SMNE). A sheep polyclonal antibody specific to SMNE was employed in the assay to capture any remaining SMNE impurities in the sample. Both the Calibration standards and test samples were simultaneously added to the microtiter plate coated with affinity purified capture antibody and followed by incubation and washing. The biotinylated antibody was added to the microtiter plate to bind the SMNE and then reacted with streptavidin labeled with HRP (Horseradish Peroxidase). TMB (3,3',5,5' -tetramethylbenzidine) substrate was added into reaction, HRP catalyzed the oxidation of TMB by H_2O_2 to produce a blue product (maximum absorption peak at 655 nm). Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the SMNE concentration in the Calibration standard and the sample. The concentration of SMNE in the sample can be calculated using a dose-response curve.

■ Kit Contents

Table 1.Kit Components

Reagent	Part No.	Quantity	Note
SMNE Calibration standard	PNB004	1 × 100 µL	Please refer to the details on the label of the tube.
Anti-SMNE Microtiter Strips	PNA004	8 well × 12 strips	Strips pre-coated with sheep anti-SMNE antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
SMNE Diluent	PNE003	2 × 25 mL	For dilution of calibration standards, biotinylated antibody, streptavidin-HRP and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	For plate washing. Dilute 10 times in freshly prepared ultra-pure water to obtain 1×Wash Buffer solution.
Anti-SMNE : Biotinylated Conjugate (100×)	PNG004	1 × 120 µL	Biotinylated anti-SMNE antibody (sheep polyclonal) in a protein matrix with preservative. Dilute 100 times in SMNE diluent before use.
Streptavidin-HRP (100×)	PNH002	1 × 140 µL	Streptavidin labeled with HRP. Sealed and keep away from light. Dilute 100 times in SMNE diluent before use.
TMB Substrate	PND003	1 × 12 mL	3,3',5,5'-tetramethylbenzidine; Sealed and keep away from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	1 M hydrochloric acid. Avoid direct contact with eyes, skin, and clothing. Wear goggles while handling.
Sealing Film	PNK001	3 pieces	Cover the strips with it during incubation to prevent contamination and liquid evaporation.

Note: Room temperature refers to 25 ± 3℃.

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as shown in Table 2.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-SMNE microtiter strips	Store in the bag with desiccant at 2-8℃ for up to 30 days.
SMNE Calibration standard	Store at 2-8℃ for up to 1 year.

■ Materials Required But Not Provided

- Sterilized centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel micropipettes.
- Vortex mixer
- Incubator (optional)
- Plate washer (optional)
- Water bath (optional)

■ Workflow

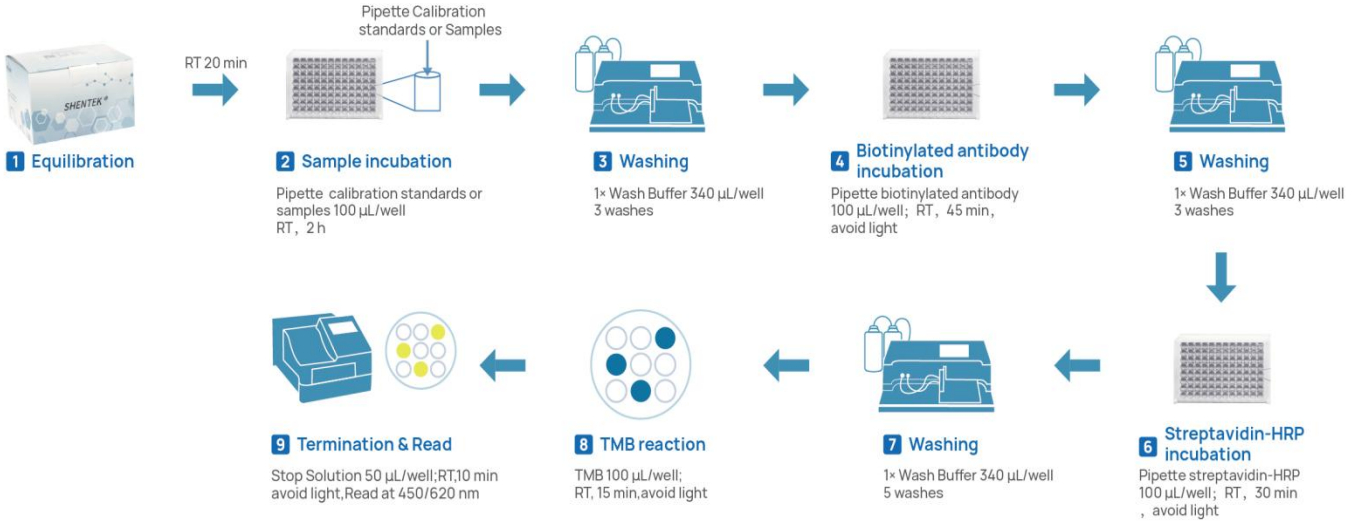


Figure 1. Procedure Flowchart

1. Preparation

(1) Equilibration

- Before use, allow the kit to equilibrate at room temperature for 20 minutes, return to 2-8℃ immediately after use.
- Take appropriate amount of strips to a strip holder according to the experiment design. Please store the remaining strips in the bag with desiccant at 2-8℃.

(2) Preparation of Reagents

- 1× Wash Buffer: Dilute 1 volume of Wash Buffer with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix well before use.

Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37 ℃ in the water bath until it clears.

(3) Preparation of Calibration standard Solutions

- Prepare SMNE Calibration standard solutions as indicated in Fig 2 and Table 3.

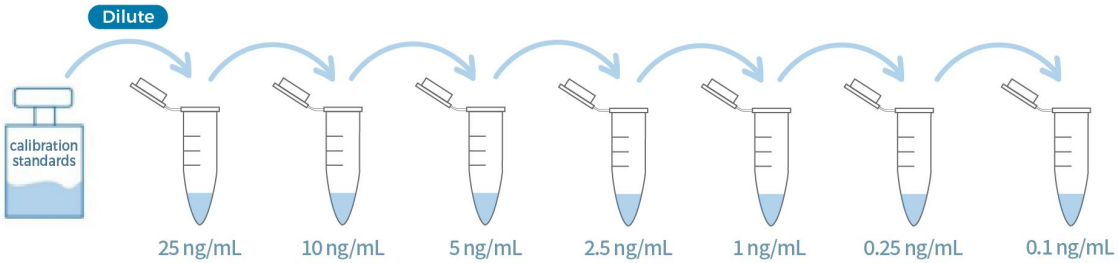


Figure 2. Graphic scheme of SMNE Calibration standard solutions

Table 3. Preparation of SMNE Calibration standard solutions

Serial Dilution Tube	Dilution procedure	Conc.(ng/mL)
ST1	Dilute the SMNE Calibration standard to ST1 with SMNE Diluent	25
ST2	400 µL ST1 + 600 µL SMNE Diluent	10
ST3	500 µL ST2 + 500 µL SMNE Diluent	5
ST4	500 µL ST3 + 500 µL SMNE Diluent	2.5
ST5	400 µL ST4 + 600 µL SMNE Diluent	1
ST6	250 µL ST5 + 750 µL SMNE Diluent	0.25
ST7	400 µL ST6 + 600 µL SMNE Diluent	0.1
NCS	SMNE Diluent	0

Note: It is recommend to dilute the SMNE Calibration standard 10 times in SMNE Diluent before use. Prepare ST1 using the 10-fold diluted calibration standard.

(4) Sample Preparation

- Test samples: In-process samples, harvested bulk, drug substance and drug product. Make sure samples are clear and transparent, and insoluble substances need to be removed by centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Avoid repeated freeze-thaw cycles. For long-term storage, -70°C or below is recommended to avoid degradation.
- Dilute the samples with SMNE Diluent to achieve a proper range of SMNE concentration within the calibration curve.
- For the first use, a method validation is recommend to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample dilution series.

Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Incubation

- Pipette 100 μL of Calibration standard, controls and samples into each designated well according to the experimental design. Avoiding foaming bubbles during pipetting. It is recommended to prepare 2-3 replicates for each concentration.
- Seal the plate and incubate for 2 hours at room temperature.

Table4. Example of the 96 tests layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B	ST7	ST7	ST7		S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5		S1+SRC	S1+SRC	S1+SRC					
E	ST4	ST4	ST4		S2+SRC	S2+SRC	S2+SRC					
F	ST3	ST3	ST3		S3+SRC	S3+SRC	S3+SRC					
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

- ✧ “ST1-ST7” indicate 7 concentration gradients, “NCS” as negative control, “S1-S3” as test samples , and “S1 SRC-S3 SRC” as the spiked recovery controls for each sample.
- ✧ The number of replicates and the spiked samples can be determined by method validation.

(2) Biotinylated Antibody Reagent Preparation and Incubation

- 1×Anti-SMNE:Biotinylated Conjugate: Prepare the 1×Anti-SMNE:Biotinylated Conjugate by diluting the Anti-SMNE:Biotinylated Conjugate (100×) with SMNE Diluent in a new centrifuge tube. Prepare 1×Anti-SMNE:Biotinylated Conjugate fresh, mix gently and use it immediately.
- Wash the plate with 340 μL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dry before adding the substrate.
- Pipette 100 μL of 1×Anti-SMNE:Biotinylated Conjugate into the corresponding wells as indicated earlier.

- Seal the plate and incubate for 45 min at room temperature, and protect from light.

(3) Streptavidin-HRP Preparation and Incubation

- 1×Streptavidin-HRP: Prepare the 1×Streptavidin-HRP by diluting the Streptavidin-HRP (100×) with SMNE Diluent in a sterile centrifuge tube. Prepare 1×Streptavidin-HRP fresh, mix gently and use it immediately.
- Wash the plate with 340 μL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 3 times. Do not allow the wells to be completely dry before adding the substrate.
- Pipette 100 μL of 1×Streptavidin-HRP into the corresponding wells.
- Seal the plate and incubate for 30 min at room temperature, and protect from light.

(4) TMB Reaction

- Equilibrate the TMB substrate for 20 min at room temperature.
- Wash the plate with 340 μL of 1×Wash Buffer per well and soak for 30 seconds. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 5 times. Do not allow the wells to be completely dried before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, and incubate at room temperature for 15 min, and protect from light.

  Note : Do not use sealing film during this step.

(5) Termination

- Add 50 μL of Stop Solution into each well.

  Note: The order of adding stop solution should be the same as the order of adding the TMB substrate. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.
- Incubate at room temperature for 10 min, protect from light.

(6) Reading

- Read absorbance at 450 nm/620-650 nm.

3. Calculation and Analysis

- The OD value of each well should be calculated by the difference between OD_{450nm} and their respective long wavelength. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- Subtract the OD value of the NCS from each calibration point and samples, and record the mean of the replicate wells.
- Perform a 4-parameter logistic regression model using the Calibration standard concentration values and OD values to obtain the calibration curve equation. Substitute the average OD value of the sample into the equation to calculate the sample concentration, which should be multiplied by the dilution factor to obtain the actual sample concentration.
- The software for data analysis of the standard curve could be the one that comes with the microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- For samples with absorbance values above the Calibration standard ST1, a pilot study should be performed to determine an appropriate dilution before retesting. The SMNE concentration in the sample is calculated from the test value multiplied by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

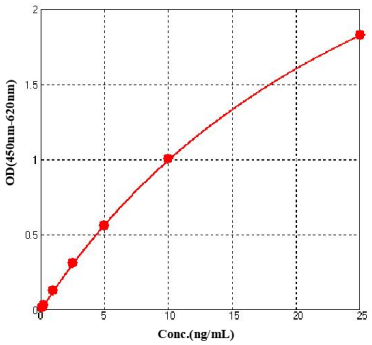
■ Limitations

- This product is intended for research use only but not for clinical use.
- It is recommended to keep the pH of the sample solution between 6.5 and 8.5, as exceeding this pH range may affect the results of the sample assay.

■ Assay Performance

- Linearity& Range: 0.1-25 ng/mL，$R^2 \geq 0.990$
- LLOQ: 0.25 ng/mL
- Specificity: No cross-reactivity with Rnase, host cell proteins (e.g. *E.coli*, CHO, HEK293T , Vero cells) and BSA.
- Typical calibration curve and data:

Calibration Standards (ng/mL)	Abs. at (450 nm-620 nm)	Mean Abs
25	1.9522	1.8645
	1.7950	
	1.8464	
10	1.0560	1.0387
	1.0208	
	1.0393	
5	0.6067	0.6001
	0.5931	
	0.6004	
2.5	0.3472	0.3492
	0.3396	
	0.3607	
1	0.1647	0.1669
	0.1653	
	0.1707	
0.25	0.0708	0.0707
	0.0711	
	0.0701	
0.1	0.0547	0.0534
	0.0531	
	0.0524	
0	0.0403	0.0382
	0.0384	
	0.0359	



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A = 4.08707

B = -1.00169

C = 30.95401

D = 0.00166

R^2 = 0.99997

Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents from different lot numbers.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate immediately after sample incubation to avoid liquid evaporation.
- ✧ Avoid drying the wells before TMB substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination..
- ✧ Centrifuge Anti-SMNE:Biotinylated Conjugate (100×) and Streptavidin-HRP (100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipette or sampling accurately for dilution of standards and samples, for example, a minimum volume of 5 μL is recommended.
- ✧ SMNE Calibration standard solutions, Biotinylated antibody solution and Streptavidin-HRP solution are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assistance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Sometimes air, resulting in bubbles, can be drawn into the micropipette or dispensed into the wells. If this happens, bubbles can influence optical density values and detection results.
- ✧ Plate reading should be completed within 30 minutes after termination.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of SMNE levels.
- ✧ Sorbitol can cause abnormal signal with high background signal. The sorbitol concentration in the sample cannot exceed 0.01%.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including ultra-pure water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including pipettes and centrifuge	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the wash buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Add the samples to the bottom of the wells using micropipettes, and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation Guidance for Industry
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples
- Gimadutdinov OA, Khamidullina RG, Fazleeva II, Trushin MV. Structure, Function and Evolution of Serratia marcescens Endonuclease. Journal of Experimental Biology and Agricultural Sciences, 2018, 6(1):53-61.
- Gousseinov E, Kools W, Pattnaik P. Nucleic Acid Impurity Reduction in Viral Vaccine Manufacturing. BioProcess International, 2014, 12(2):59-59.

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Support & Contact

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