Ultra Nuclease ELISA Kit - Instruction

Catalog: ARK6624

【Intended use】

The kit is used for quantitative detection of Ultra Nuclease in intermediate, semi-finished and finished of various biological products.

【Principle】

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues of Ultra Nuclease in the samples. The capture antibody is coated with 96-well labeling plate and the solid phase antibody is prepared. Then first add the standard and testing samples, and second put a marker on horseradish peroxidase labeled antibody, forming a solid phase antibody - Ultra Nuclease- labeled antibody sandwich, reaction after washing, then add the chromogenic reaction of the substrate. The substrate under the catalysis of HRP become blue, and under the action of stop solution into the final yellow. The absorbance (OD value) is measured at 450nm, and the content of Ultra Nuclease in the sample to be tested is calculated by the standard curve.

【Materials provided】

Number	Component	96T	Storage condition
1	Microwell Plate	8×12	2-8℃
2	Standard（0.5μg/mL）	300μL×1	2-8℃
3	HRP-Conjugate Antibody	15mL×1	2-8℃
4	Sample Dilution	30mL×1	2-8℃
5	Wash Buffer (20×)	30mL×1	2-8℃

6	Developing solution A	8mL×1	2-8℃ (keep in dark)
7	Developing solution B	8mL×1	2-8℃ (keep in dark)
8	Stop Solution	15mL×1	2-8℃
9	Microplate Sealers	3	-

【Storage】

Unopened Kit store at 2-8℃ and the kit is stable for 12 months.

【Reagents/equipments required but not supplied】

1. Enzyme-labeled instrument, label plate thermostatic oscillator or thermostatic incubator, microplate Washer
2. Pipettes and pipette tips (0.5- 10μL, 10- 100μL, 30-300μL, 100- 1000μL)
3. Deionized or distilled water, bibulous paper, EP tubes

【Reagent preparation】

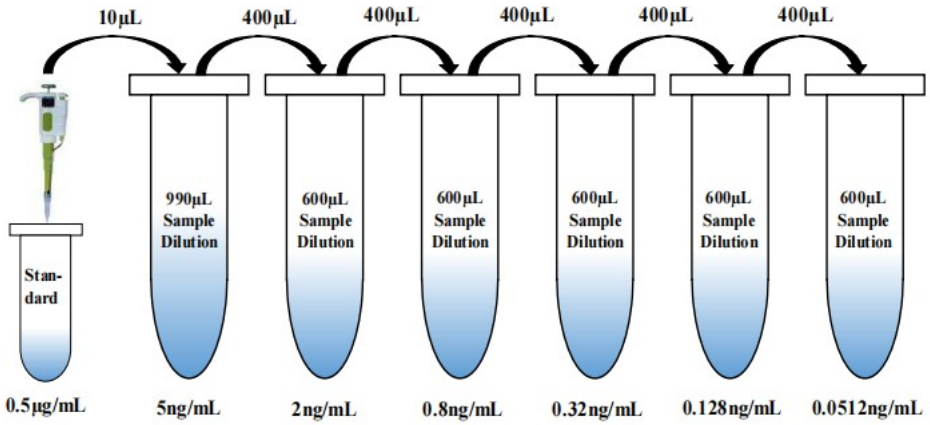
- Wash Buffer(1×):Take 1 part of Wash Buffer (20×) and add 19 parts of deionized water to prepare a working concentration wash buffer (1×). If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- Standard preparation: Standards are diluted to 5 ng/mL with sample dilution and then prepared by 2.5 times the dilution method.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.

【Assay procedure】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃.

2. Preparation of the standard curve: dilute the Ultra Nuclease standard with sample dilution to 5ng/mL, then prepared by 2.5 times the dilution method.



3. Temperature breeding of samples: setup standard wells, blank wells and sample wells.

100μL of standard substances of different concentrations were added to the standard wells in order, and 100μL of sample dilution to the blank wells, and 100μL of samples to be tested were added to the sample wells, and then incubated at 37 ℃ for 1h.

4. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5s can be slightly shaken after adding wash buffer).

5. HRP-conjugate antibody incubate: Add 100 μL of HRP-conjugate antibody each well, and then incubate at 37℃ for 1h.

6. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250μL /well). Pat dry residual liquid in sample well.

7. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100μL /well), and incubated in darkness for 15min.

8. Termination: add stop solution (100μL /well).

9. Recording: The OD value at 450/630nm should be measured by placing the plate in the marker, and the reading should be completed within 20min.

【Calculation of results】

If samples generate values higher than the highest standard, dilute the sample and repeat the assay.The average OD value of each standard product minus the OD value of the blank well as the correction value. The OD450nm value of the Ultra Nuclease standard was taken as the dependent variable Y, and the standard concentration was taken as the independent variable X to make the curve. Werecommend to use four-parameter fitting equation: Y=((A-D)/(1+(x/C)^B))+D. The absorbance value of the sample OD450nm was substituted into the formula to calculate the Ultra Nuclease content of the sample.

【Precautions】

1. This kit is only used for research in vitro study and is not for use in clinical diagnosis.
2. This kit should not be used beyond the expiration date.
3. All reagents in the kit must be restored to room temperature (20-25℃) before use.
4. The best detection effect can be guaranteed only by strictly following the operation method of the instructions and using all the reagents matched with the kit.
5. Each test should prepare the corresponding standard curve. DO NOT USE the standard curves from different kits or different batches.

6. Pay attention to the timely replacement of sample adding tank and pipette tips between different samples and steps to avoid cross contamination.

7. The final test results are closely related to the effectiveness of the reagent the operator's method of operation and the test environment.

8. The company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Users are requested to take full account of the possible usage of the sample and reserve sufficient samples before using the kit.

【Safety instructions】

1. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

2. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

3. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

【Problem analysis】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Standard curve gradient bad	Suction or addition of liquid is inaccurate	Check pipette and tips
	Standard is not properly diluted	Configure the standard curve according to the kit instructions

	Enzymatic plate washing is incomplete	Ensure the frequency of washing plate and the amount of wash buffer per well
Color is very weak or colorless	The incubation time is too short	Ensure adequate incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent is insufficient or missing in volume	Check the suction and addition process to ensure that all reagents are added in sufficient quantities in order
	Incorrect dilution	
Low reading of OD	The setting of the enzyme marker is incorrect	Check the wavelength and filter device on the marker
		Open the micrometer in advance for preheating before reading
Large coefficient of variation	Add reagents incorrectly	Check the filling condition
	Contamination at the bottom of the labeling plate	Check the bottom of the plate for any remaining liquid and fingerprints
	Foreign matter or air bubble in plate well	Confirm that there is no foreign matter in the plate hole before adding sample, and confirm that there is no bubble after adding sample
High background values	Detection the concentration of antibody	Use the recommended dilution
	Enzymatic plate washing is incomplete	Wash the board as recommended in the instructions
		If automatic plate washer is used, check all feed and drain orifices for clogging
		If the board is washed by hand,the number of times can be increased appropriately
	The wash buffer is contaminated	Prepare fresh wash buffer
Weak sense	The kit was not properly	Keep the relevant reagents according to

	stored	the instructions
	Not terminated before reading	A stop solution should be added to each well before reading OD