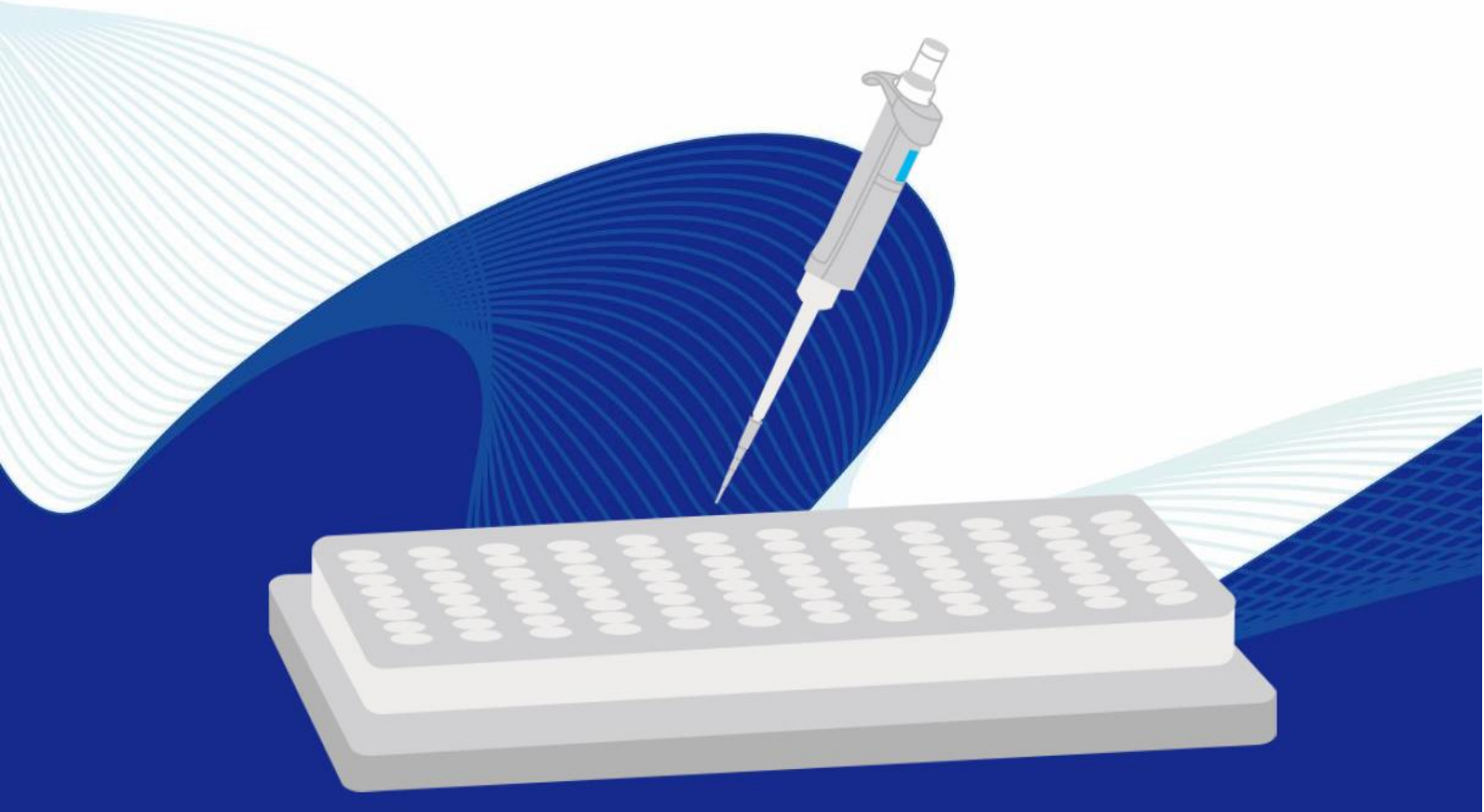
Intelli Nuclease ELISA Kit

Protocol Booklet



Product Name

Intelli Nuclease ELISA Kit

Specification

96T/kit

Intended Purpose

The kit is for quantitative detection of the content of omnipotent nuclease in samples.

Principle of Test

The kit is designed on the basis of double antibody sandwich principle. The wells of its ELISA plate are coated with anti-omnipotent nuclease antibody. Upon adding the samples for inoculation and washing, adding horseradish peroxidase (HRP)-labeled antibody for inoculation, antibody-antigen-enzyme-labeled antibody complex is formed. After thorough washing, the substrate TMB is added to develop color which converts to blue under the catalysis of peroxidase, and finally converts to yellow by acid termination. The depth of color is positively correlated with the content of omnipotent nuclease in the sample. The absorbance (OD value) is measured with a microplate reader at a wavelength of 450 nm, and the concentration of omnipotent nuclease in the sample is calculated according to the calibration curve.

Composition of Kit

Name	Specification	Qty
Pre-coated Microplate	8x12 wells	1
Enzyme conjugate (100X)	0.15mL	1
Diluent	30mL	1
Chromogenic solution	6mL	2
Stop solution	6mL	1
Concentrated wash buffer (10X)	50mL	1
Concentrated standard	0.5mL	1
Plate sealer		4 sheets
Protocol booklet		1 copy

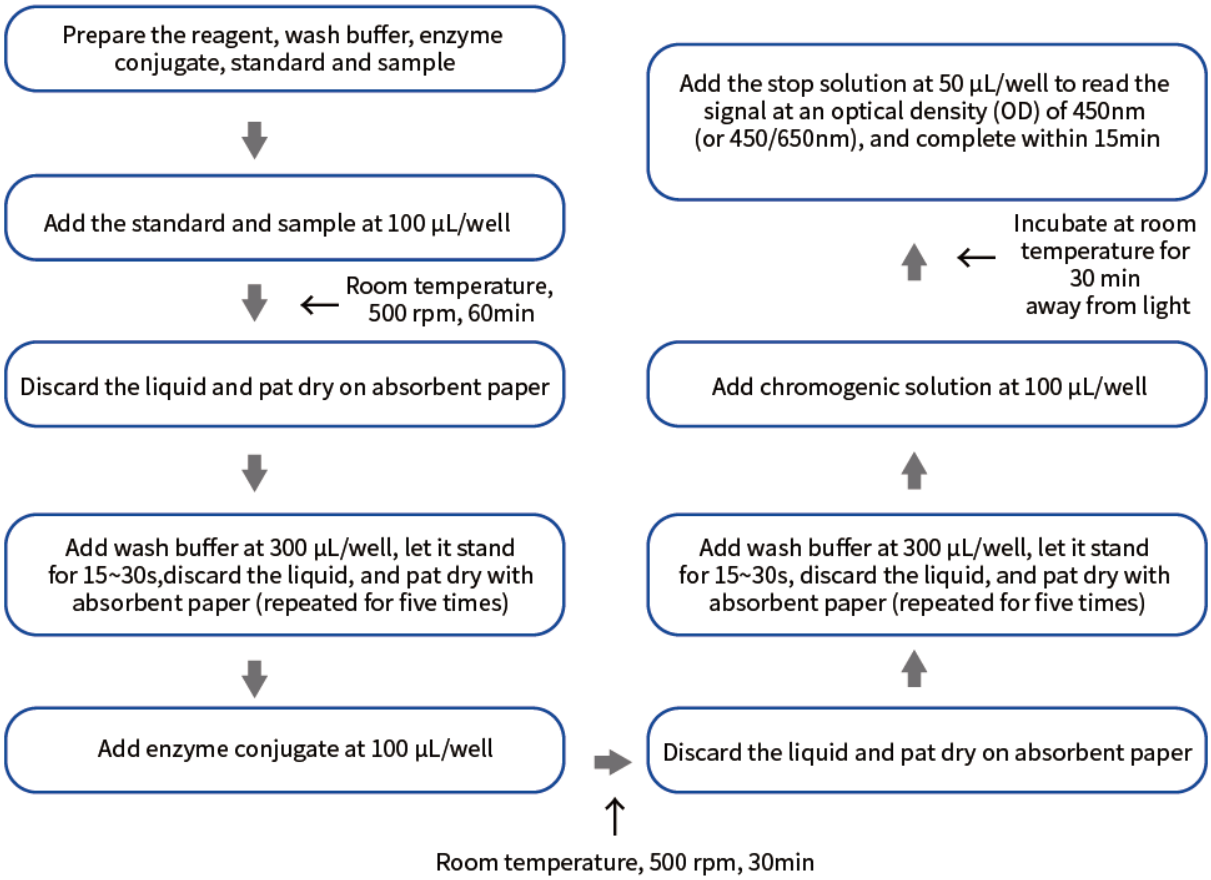
Storage Condition and Stability

1. Store at 2~8℃ for 12 months.
2. Please use up within 6 weeks after unpacking.

Instruments Required

1. Microplate reader (with filter at 450 nm, recommended to include dual wavelength detection mode, with primary and secondary wavelength at 450 nm and 650 nm, respectively).
2. Plate shaker (400-600 rpm).

Flow Chart



Preparation of Regents

Equilibrate the kit to room temperature (18~25C).

Dilute 10x concentrated wash buffer with purified water at a volume ratio of 1:9 to obtain the wash working solution.

Dilute the 100× enzyme conjugate 100-fold with diluent before use.

Dilute the concentrated standard (1000 ng/mL) with diluent to 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL,1.25 ng/mL, 0.63 ng/mL, 0.31 ng/mL, O ng/mL.

The recommended dilution method is as follows

	Final conc.	Dilution method	
NO.	(ng/mL)	Diluent	Spike amount of working standard
A	20	0.98mL	20 µL concentrated standard
B	10	0.5mL	0.5 mL A solution
C	5	0.5mL	0.5 mL B solution
D	2.5	0.5mL	0.5 mL C solution
E	1.25	0.5mL	0.5 mL D solution
F	0.63	0.5mL	0.5 mL E solution
G	0.31	0.5mL	0.5 mL F solution
H	0	0.5mL	/

Assay Procedures

1. Remove the desired strips from the aluminum foil bag equilibrated at room temperature, and seal the remaining strips in a Ziplock bag and put them back at 2-8°C.
2. Set the standard wells and sample wells. It is recommended that all standards and samples to be tested in double wells. Add 100 μL of standard at difference concentration to each standard well, and add 100 μL of sample to be tested to the sample wells.
3. Seal the reaction wells with the pleat sealer and shake the plate (at 500 rpm) for 60min at room temperature.
4. Discard the liquid, pat dry on absorbent paper, fill each well with wash buffer (300 μL), let stand for 15-30s, shake off the wash buffer, pat dry on absorbent paper, and repeat the plate washing 5 times.
5. Add 100 μL of enzyme conjugate at working concentration to each standard well and sample well, seal the reaction wells with the plate sealer, and shake the plate (at 500 rpm) for 30min at room temperature.
6. Discard the liquid, pat dry on absorbent paper, fill each well with wash buffer (300 μL), let stand for 15-30s, shake off the wash buffer, pat dry on absorbent paper, and repeat the plate washing 5 times.
7. Add 100 uL of chromogenic solution to each well, seal the reaction wells with the plate sealer, and let stand at room temperature for 30min away from light.
8. Add 50 μL of stop solution to each well for detection within 15min, and set the wavelength of the microplate reader at 450nm (dual wavelength 450 nm/650 nm is recommended).

Interpretation of Test Results

1. The calibrated OD values of standard and samples are calculated by take the average optical density (OD) values of standard, blank control and samples, and subtracting the average OD values of the blank control. A calibration curve is plotted with the concentration of the standard as the abscissa and the OD value of calibrated standard as the ordinate. (The OD value in the dual wavelength detection mode is 450 nm minus 650 nm.)
2. A variety of graphing and statistical software are available to aid in the development of calibration curves and the calculation of unknown sample concentration. The cubic curve fitting method usually has a satisfactory fitting effect, and other methods such as Logistic (4P) may also achieve such a result. Therefore, applicable method needs to be analyzed according to the specific experimental data.

Test Performance

1. LOD: 0.06 ng/mL
2. LLOD: 0.2 ng/mL
3. Linearity range: 0.2-20 ng/mL
4. Precision: Intra-run CV<10%, inter-run CV<15%
5. Recovery: 80%-120%
6. Specificity: The kit is used with Duoning Intelli Nuclease. It can also be used for detection of omnipotent nuclease from other manufacturers, but corresponding products should be used to establish a calibration curve.

Cautions

1. The color development temperature and time are vital to the experimental results and should be accurately controlled.
2. During the washing process, the reaction plate should be soaked in the wash buffer for 15-30s and then dried

to fully wash the non-specifically absorbed components.

3. All reagents should be shaken well before use. The spiked samples should be added to the bottom of the wells of ELISA plate to avoid adding them to the upper part of the wall. Be careful not to splash or generate bubbles.
4. If crystals are found in the concentrated wash buffer, incubate in a water bath at 37°C, and then mix and dilute to the working solution after the crystals are complete dissolved.
5. Sodium azide (NaN_3) should not be added to the sample as it may destroy the activity of horseradish peroxidase, resulting in a lower detection value.

Technical Support

Please contact Duoning Biotechnology for any technical questions and supports at: marketing@duoningbio.com.

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