Ultra Nuclease Assay Kit (ELISA)

Cat.No.: HYI133

Product Description

This UltraNuclease ELISA kit is a sandwich ELISA to be performed in a microplate format. Sample potentially containing endonuclease are incubated in microtiter plate wells which have been pre-coated with an affinity purified anti-endonuclease capture antibody. After incubation and a washing step in which unbound components are removed, a biotin-conjugated, anti-endonuclease detector antibody is added. After further washing steps, the bound detector antibody reacts with an enzyme conjugate acting as tracer. After a final washing step, a substrate solution is added to the wells and reacted, resulting in color development. The optical density is measured photometrically and is proportional to the analyte concentration present in the wells. The endonuclease concentration in unknown samples can be calculated based on the corresponding standard curve.

Components

Reagent	96 T
ELISA microplate	8×12 wells
Detector antibody (100×)	0.15mL×1
Enzyme conjugate (1000×)	15μL×1
Dilution buffer	50mL×1
Substrate solution	12mL×1
Stop solution	6mL×1
Wash concentrate (10×)	60mL×1
Standard concentrate	0.1mL×1
Sealing film	4 films

Note: Control standard endotoxin is freeze-dried powder. See label for titer and reconstitution volume.

Storage and stability

Unopened kit should be stored at 2-8℃ for stability until the expiration date printed on the kit. Opened reagents should be stored at 2-8℃ and are stable for 6 weeks.

Equipment required

1. Microplate reader spectrophotometer with dual wavelength capability at 450 and 650nm. (If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)
2. Microtiter plate rotator (400-600 rpm).

Instructions

1. Reagent preparation

Bring all reagents to room temperature (23℃±2℃) before use.

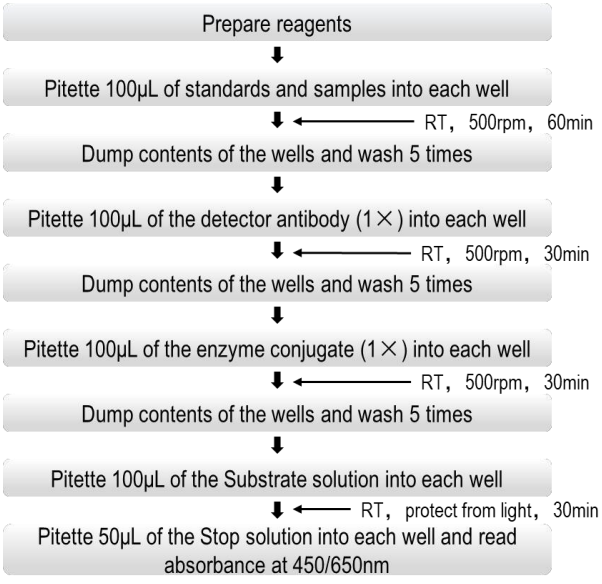
1) Prepare washing buffer (1×) by diluting 50mL of the Wash concentrate (10×) with 450mL of deionized or distilled water.
2) Detector antibody (1×) : Centrifuge the Detector antibody (100×) at 7000rpm for 20seconds. Dilute to work concentrate at 1:99 by dilution buffer before use.
3) Enzyme conjugate (1×) : Centrifuge the Enzyme conjugate (1000×) at 7000rpm for 20 seconds. Dilute to work concentrate at 1:999 by dilution buffer before use.
4) Dilute the Standard concentrate (1000ng/mL) with dilution buffer to 2ng/mL, 1ng/mL, 0.5ng/mL, 0.25ng/mL, 0.125ng/mL, 0.063ng/mL, 0.031ng/mL, 0ng/mL. Standards(2ng/mL to 0ng/mL) are prepared according to the following dilution scheme:

ID	Concn. (ng/mL)	Dilution buffer	Reagent
A	2	0.998mL	2μL of Standard concentrate
B	1	0.5mL	0.5mL of A
C	0.5	0.5mL	0.5mL of B
D	0.25	0.5mL	0.5mL of C
E	0.125	0.5mL	0.5mL of D
F	0.063	0.5mL	0.5mL of E
G	0.031	0.5mL	0.5mL of F
H	0	0.5mL	/

2. Assay procedure

1) Take out the required strips form the foil of the ELISA microplate after reaching room temperature. Remaining plate strips not used in the assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.
2) Pipette 100μL of standards (2ng/mL to 0ng/mL) or samples into each well.
3) Cover and incubate on shaker at 500rpm for 60 min at room temperature.
4) Dump contents of the wells. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Fill wells generously with 300μL/well of washing buffer (1×). Dump and tap again. Repeat for a total of 5 washes.
5) Pipette 100μL of the detector antibody (1×) into each well. Cover and incubate on shaker at 500rpm for 30 min at room temperature.
6) Dump contents of the wells. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Fill wells generously with 300μL/well of washing buffer (1×). Dump and tap again. Repeat for a total of 5 washes.
7) Pipette 100μL of the enzyme conjugate (1×) into each well. Cover and incubate on shaker at 500rpm for 30 min at room temperature.
8) Dump contents of the wells. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Fill wells generously with 300μL/well of washing buffer (1×). Dump and tap again. Repeat for a total of 5 washes.
9) Pipette 100μL of the Substrate solution into each well. Cover and incubate for 30 min at room temperature. Protect from light. (DO NOT SHAKE.)
10) Pipette 50μL of the Stop solution into each well. Read absorbance at 450/650nm within 15 min.

Assay flowchart



Calculation of result

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Absorbances of samples are then interpolated from this standard curve.
2. Most graphing software can help make the curve and cubic usually provide the best fit, though other equations (e.g. 4 parameter logistic fit) can also be tried to see which provides the most accurate.

Performance characteristics

1. Lower limit of detection: 0.01 ng/mL
2. Lower limit of quantitation: 0.02 ng/mL
3. Linearity: 0.02~2 ng/mL
4. Precision: Intra assay CV ≤10%, Inter assay CV ≤15%
5. Recovery: 80%~120%
6. Specificity: This immunoassay is calibrated against UltraNuclease, available from Hyasen Biotechnology Co., Ltd. Endonuclease from other suppliers may show a different reactivity with regard to sensitivity and accuracy, therefore the compatibility of the kit calibration to the individual endonuclease product must be verified.

Notes

1. TMB reaction temperature and time is critical, please control them according to the instruction strictly.
2. Thorough washing is essential to proper performance of this assay. The manual method described in the assay procedure is preferred for best precision, sensitivity and accuracy.
3. All reagents should be shaken well before use. When adding samples, add the sample to the bottom of the well of the ELISA plate, avoid adding to the upper part of the well wall, and be careful not to splash or generate bubbles when adding samples.
4. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
5. Avoid the assay of samples containing sodium azide (NaN_3) which will destroy the HRP activity of the conjugate and could result in the underestimation of UltraNuclease levels.