XhoI ELISA Kit

REF: EG24602S

Storage Condition

Stored at 2~8℃ . Keep away from light.

Components

Component	Amount
XhoI Standard	10 μg, 2 Vial
XhoI Detection Antibody (HRP labeled)	12 ml, 1 Vial
TMB Substrate	12 ml, 1 Vial
Stop Solution	12 ml, 1 Vial
Sample Diluent	50 ml, 1 Vial
Wash Buffer (20×)	50 ml, 1 Vial
Microtiter Plate (Coated)	96-well, 1 Plate

Description

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for XhoI has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any XhoI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for XhoI is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of XhoI bound in the initial step. The color development is stopped and the intensity of the color is measured. And the residual degree of XhoI in the sample is measured.

Detection range: 0.125~4 ng/ml

Quantification limit: 0.125 ng/ml

Detection limit: 0.021 ng/ml

Equipment

Microplate reader, microplate shaker, vortex mixer, pipette, sealing film, etc.

Reagent Preparation

1. Bring the required reagents to room temperature (25 ± 3℃) before use. Determine the number of wells to be used for the assay, remove the required number of microplate strips, reseal the remaining strips, and store at 2~8℃ .

2. 1× Wash Buffer Preparation: Based on the volume of wash buffer required for this experiment, measure an appropriate amount of 20× wash buffer and dilute with deionized water at a 1:20 volume ratio, mix well, and set aside for use.

3. Standard Solution Preparation (Example):

Accurately add 1 ml of sample diluent to XhoI standard, mix well, the final concentration is 10000 ng/ml. The prepared standard solution should be stored at 2~8℃ and used within 24 hours. If it is not used for a long time, it needs to be stored at -20℃ and used within 3 months.

Number	Concentration of standard solution（ng/ml）	Volume of standard solution（μl）	Volume of diluent (μl)	Total volume (μl)	Final concentration（ng/ml）	Remaining volume (μl)
StA	10000	20	1980	2000	100	1980
St7	100	20	480	500	4	250
St6	4	250	250	500	2	250
St5	2	250	250	500	1	250
St4	1	250	250	500	0.5	250
St3	0.5	250	250	500	0.25	250
St2	0.25	250	250	500	0.125	500
St1(Blank)	/	/	300	300	0	300

Add: 28 Yuehu Road, Lianyungang Area,China (Jiangsu) Pilot Free Trade Zone.
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Experimental Procedure

1. Prepare all reagents and working standards as directed in the previous sections.

2. Add 100 µl of standard solution (St1~St7) or the test sample per well. Seal the plate with a cover or sealing film and incubate at room temperature (25 ± 3℃) with shaking at 400~500 rpm for 1 hour.

3. Discard the liquid from the wells, add at least 350 µl of 1× wash buffer to each well, wash each well, and tap the plate gently on clean paper to dry. Repeat this process three times. (Note: Do not let the plate wells dry excessively for a long time. Improper washing may lead to a higher CV value between replicate wells, and signal bias either high or low).

4. Add 100 µl of XhoI detection antibody (HRP-labeled) solution to each well. Seal the plate with a cover or sealing film and incubate at room temperature (25 ± 3℃) with shaking at 400~500 rpm for 30 minutes.

5. Discard the liquid from the wells, add at least 350 µl of 1× wash buffer to each well ,wash each well, and tap the plate gently on clean paper to dry. Repeat this process three times. (Note: Do not let the plate wells dry excessively for a long time).

6. Add 100 µl of TMB substrate to each well and incubate at room temperature (25 ± 3℃) for 20 minutes.Protect from light.

7. Add 100 µl of stop solution to each well, gently tap the plate to mix and stop the reaction.

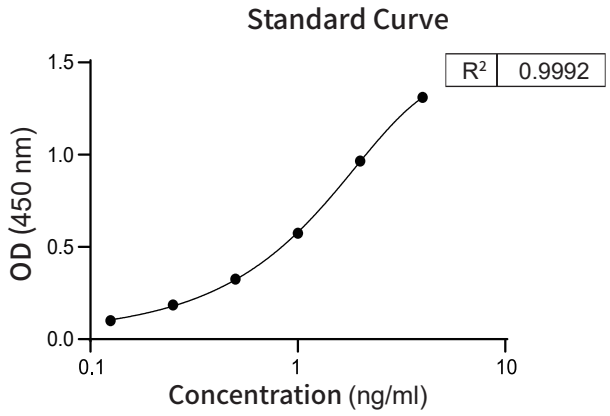
8. Read the absorbance at a wavelength of 450 nm within 10 minutes after adding the stop solution.

Result Calculation

It is recommended to generate a four-parameter curve with the log of the BsaI concentrations versus the O.D.

Table 1 Typical Standard Curve Data Table

Concentration of standard solution (ng/ml)	Absorbance		
	Measurement 1	Measurement 2	Mean
4	1.432	1.410	1.421
2	1.056	1.087	1.071
1	0.694	0.683	0.689
0.5	0.439	0.435	0.437
0.25	0.298	0.289	0.293
0.125	0.202	0.218	0.210
0	0.113	0.110	0.111



Typical standard curve plot

Performance

Detection Limit: 0.021 ng/ml.

Quantification Limit: 0.125 ng/ml.

Linearity: $R^2 \geq 0.98$.

Repeatability: testing three known concentration samples 20 times.

Intermediate Precision: testing three known concentration samples three times at different times and by different person.

Name	Repeatability (n=20)			Intermediate Precision (n=3)		
	Low	Medium	High	Low	Medium	High
Mean (ng/ml)	0.46	1.99	3.05	0.53	2.09	3.31
SD	0.017	0.079	0.259	0.025	0.049	0.255
CV (%)	3.7	4.0	8.5	4.7	2.3	7.7

Recovery: Assesses the recovery of XhoI added to different levels within the entire range of the assay in relevant matrices.

Average recovery (%) (n=3)	Recovery range (%)
105.9	96.2 ~ 117.6

Notice

1. This product is for research use only and is not intended for clinical diagnosis or treatment.

2. Reagents should be stored according to the conditions specified on the label and should be equilibrated to room temperature before use.

3. The pre-coated microplate should be equilibrated to room temperature before opening the packaging, and any remaining strips should be immediately returned to the packaging, resealed, and stored at 2~8℃ . Remaining reagents should also be resealed and stored according to the conditions specified on the label.

4. XhoI standard solution and XhoI detection antibody (HRP-labeled) solution should be mixed well before use.

5. The residual liquid in the plate wells during the washing process should be dried by tapping on clean paper. The plate wells should not be excessively dried for a long time, as improper washing may lead to higher CV values between replicate wells or higher absorbance values for the blank (0 ng/ml), affecting result accuracy.

6. The TMB substrate should be a colorless, transparent liquid. If it appears light blue, it indicates that the solution has been contaminated and is not suitable for subsequent analysis.

7. Samples with extreme pH values (<5.0 or >8.5), or containing high salt, high polysaccharides, high urea, high organic solvents, and high detergent solutions, may lead to lower recovery rates.

8. Due to the complexity of biological sample matrices, it is recommended to include positive control test samples in each experiment to ensure the accuracy of the test and to use replicate wells for the test.

Safety Precautions

1. The stop solution is an acidic solution, and caution should be exercised when handling it.

2. Operators should wear personal protective equipment, such as lab coats, gloves, masks, and safety goggles.

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