BSA ELISA Kit
Catalogue Number: OM700060
(96Tests)
Store all reagents at 2-8°C
Assay range ： 1 - 32 ng/mL

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

This Exenatide ELISA kit is intended for laboratory research use only and is not to be used for diagnostic or therapeutic procedures. The Stop Solution changes the color of the solution from blue to yellow, after which the color intensity is measured spectrophotometrically at a wavelength of 450 nm. To determine the the concentration of Exenatide in the samples, this Exenatide ELISA Kit is provided with a set of calibration standards. The calibration standards are assayed at the same time as the samples, allowing the operator to plot a standard curve of optical density versus the corresponding Exenatide concentration. By comparing the optical density of the samples to this standard curve, the concentration of Exenatide in the samples can be determined.

PRINCIPLE OF THE ASSAY

This quantitative Bovine Serum Albumin (BSA) enzyme-linked immunosorbent assay (ELISA) kit utilizes a pair of anti-BSA monoclonal antibodies as the capture and detection antibodies, respectively, forming a sandwich ELISA detection kit. Referenced against the BSA standard from the United States Bureau of Standards, this kit can detect BSA as low as 1 ng/mL.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the

label.

1. Monoclonal Anti-BSA Coated plate	96 wells	1 plate
2. BSA internal reference (20 ng/mL)	0.4mL	1 vial
3. BSA standards	0.4 mL	7 vial
(32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, 2 ng/mL, 1 ng/mL, 0 ng/mL)		
4.Anti-BSA labeled HRP	0.13mL	1 vial
5. 10x Assay Buffer	30 mL	1 tube
6. Antibody diluent	13.0 mL	1 tube
7. Sample Diluent	13.0 mL	1 tube
8. TMB Substrate	1 set (A and B, 6.5 mL each)	
9. Stop Solution	13 mL	1 tube
10. Instruction	1 copy	

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader (wavelength 450 nm), pipettes (10 µL, 100 µL, 1 mL), multichannel pipette (300 µL), pipette tips, 37°C constant temperature water bath.

PRECAUTIONS

1. If the BSA concentration in the sample is higher than 32 ng/mL, dilute the sample appropriately.
2. If the sample is lyophilized, dissolve and mix thoroughly with the sample diluent.
3. During the reaction, place the entire plate in a clean, enclosed space to prevent foreign matter from falling into the wells.
4. After washing and patting dry, remove any residual bubbles from all wells.
5. After opening, return unused strips to the sealed plastic bag with desiccant and store tightly closed at 4℃.
6. During the procedure, strictly avoid contamination of pipette tips, wash bottles, vessels, etc., by BSA-containing environments or items.

ASSAY PROCEDURE

1. Dilute the concentrated wash buffer 10-fold with distilled water.
2. Remove the anti-BSA coated 96-well microplate and allow it to equilibrate to room temperature. For each standard concentration, add 50 µL/well in duplicate to the plate. Add the test samples and internal reference, each in duplicate, 50 µL/well. Then dilute the enzyme-labeled antibody 1:100 with the enzyme-labeled antibody diluent and add 100 µL/well to each reaction well (prepare the diluted enzyme-labeled antibody immediately before use, as needed). Complete the addition process within 15 minutes.
3. Incubate at room temperature (20–24°C) for 1 hour in the dark. Wash four times with wash buffer and pat dry.

4. Add 100 µL/well of TMB substrate chromogenic solution using a pipette (mix solutions A and B in equal volumes before use and bring to room temperature). Incubate at room temperature (20–24°C) for 10 minutes in the dark. Do not shake.
5. Add 100 µL/well of stop solution using a pipette, and shake gently for 5 seconds to mix.
6. Read the absorbance at 450 nm using a microplate reader.

TYPICAL DATA

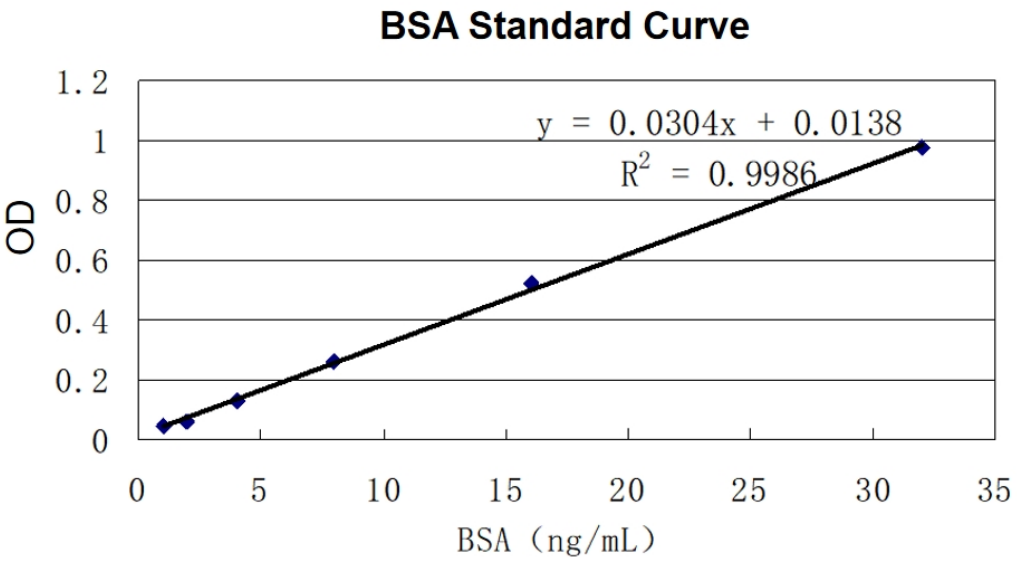
CALCULATION OF RESULTS

1. The experimental results must meet the following validity criteria:
0 ng/mL OD value ≤ 0.1; 32 ng/mL OD value ≥ 0.80; Positive reference 18–22 ng/mL; $R^2 \geq 0.98$.

2. Calculation:

In Excel, create a scatter plot with the standard concentration as the X-axis and the blank-subtracted OD value as the Y-axis. Add a trendline, then display the linear equation and R^2 value. Substitute the blank-subtracted OD value of the test sample into the linear equation to calculate the corresponding concentration.

Standard Conc. (ng/mL)	OD1	OD2	OD3	Average	Blank-subtracted
0	0.069	0.062	0.064	0.065	0
1	0.111	0.109	0.113	0.111	0.046
2	0.13	0.127	0.131	0.129	0.064
4	0.191	0.192	0.193	0.192	0.127
8	0.332	0.319	0.327	0.326	0.261
16	0.583	0.588	0.596	0.589	0.524
32	1.039	1.025	1.054	1.039	0.974



3. Determination:

In the standard curve, define:

Interval A: OD corresponding to 0–16 ng/mL
Interval B: OD corresponding to 16–32 ng/mL
Interval C: OD greater than that for 32 ng/mL

1) If the measured OD value is less than the OD value corresponding to 2 ng/mL in the standard curve, report the BSA content as <2 ng/mL.
2) If the OD values of samples at different dilutions all fall within Interval A, use the measurement from the lowest dilution for the final result calculation.
3) If measurements at different dilutions all fall within Interval B, or simultaneously within Intervals A and B, or simultaneously within Intervals B and C, use the measurement closest to the OD value for 16 ng/mL.
4) If measurements at different dilutions all fall within Interval C, further dilute the sample and re-measure to obtain an OD value within the range corresponding to 2–32 ng/mL. Calculate according to the above rules 7.3.2–4.
5) If the OD value at a higher dilution is greater than that at a lower dilution or the undiluted sample, it may indicate an operational error or the HOOK effect due to excessively high BSA concentration. Repeat the test or adjust the dilution factor.
6) Consider the dilution factor in the final result (Actual BSA content = Calculated value × Dilution factor).

Kit Technical Specifications

1. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD: Zero concentration standard plus two times the standard deviation (calculated from 20 zero-concentration wells). The LOD for this kit is calculated as 0.2 ng/mL (ppb). LOQ: Lower limit of the standard curve, 1 ng/mL.

2. Accuracy and Precision

Intra-assay and inter-assay coefficients of variation (CV%) were calculated. For intra-assay CV%, five replicates were tested at high, medium, and low concentrations (20, 10, 5 ng/mL). For inter-assay CV%, the test was repeated three times on different days. All CV% were less than 10%.

Inter-Assay				Intra-Assay			
Concentration (ng/mL)	SD	Recovery %	CV %	Concentration (ng/mL)	SD	Recovery %	CV %
20	0.06	107.3	5.6	20	0.047	102.6	4.6
10	0.049	105.4	4.7	10	0.022	101.2	2.2
5	0.059	99.66	5.9	5	0.028	97.6	2.9

3. Cross-Reactivity

This kit shows no cross-reactivity with Human Serum Albumin, Mouse Serum, Rabbit Serum, or Fish Gelatin. It shows 10% cross-reactivity with Goat Serum (calculated as the percentage ratio of the BSA concentration to the Goat Serum concentration yielding the same OD value).

Substances with No Cross-Reactivity to BSA	Reaction Concentration
Human Serum Albumin	10 mg/mL
Mouse Serum	10%
Rabbit Serum	10%
Fish Gelatin	1%

4. Hook Capacity

The HOOK effect occurs when the sample concentration is significantly higher than the highest concentration of the standard curve, resulting in a reaction OD value lower than that for 32 ng/mL BSA. For this kit, the HOOK effect occurs at concentrations above 500 μg/mL