# BSA ELISA Kit

SKU: **GXEL00386**

## Description

This kit is a quantitative sandwich ELISA for the detection of Bovine Serum Albumin (BSA). A monoclonal anti-BSA antibody is pre-coated onto the microplate as the capture antibody. Samples and standards are added, and BSA present in the sample binds to the immobilized antibody. After washing, an HRP-conjugated monoclonal anti-BSA detection antibody is added to form a sandwich complex. The signal is developed using TMB substrate and measured at 450 nm. The BSA standard is calibrated against a reference standard traceable to the National Institute of Standards and Technology (NIST). The minimum detection limit of this kit is 1 ng/ml.

## Materials Provided & Storage Conditions

| PART | DESCRIPTION | FORMAT |
|---|---|---|
| Pre-coated Microplate | 96-well polystyrene microplate (12 strips × 8 wells) pre-coated with anti-BSA capture antibody | 1 plate |
| BSA standard | 32, 16, 8, 4, 2, 1, 0 ng/ml | 1 vial each (0.4 ml/vial) |
| BSA reference | 20 ng/ml | 1 vial (0.4 ml/vial) |
| Color Reagent | Substrate Reagent A and Reagent B | 6.5 ml each |
| Stop solution | 2N H SO 2 4 | 1 vial (13 ml) |
| Standard Diluent | For dilution of standards and samples | 1 vial (30 ml) |
| Wash buffer (10x) | 10×concentrated buffered surfactant solution with preservative; dilute 1:10 with deionized water before use | 1 vial (30 ml) |
| Antibody Diluent | For dilution of detection antibody | 1 vial (13 ml) |
| Detection A | HRP-conjugated anti-BSA antibody | 1 vial (0.13 ml) |
| Instruction manual | ELISA kit instruction manual | 1 copy |

For research use only

## Storage

Store all components of this kit at the temperatures indicated on their respective labels. The kit is stable for 6 months when stored as instructed.

## Required Equipment

Microplate reader (450 nm); adjustable micropipettes (10 ul, 100 ul, and 1 ml); 300 ul multichannel pipette; pipette tips; 37°C water bath.

## Assay Procedure

1. Dilute the 10× concentrated wash buffer 1:10 with distilled or deionized water before use.

2. Remove the pre-coated anti-BSA 96-well microplate and allow it to equilibrate to room temperature. Add 50 ul per well of BSA standards in duplicate. Add 50 ul per well of test samples and internal reference, each in duplicate. Dilute the HRP-conjugated detection antibody 1:100 with antibody diluent, then add 100 ul per well to each well. Prepare only the required volume immediately before use. Complete all sample and reagent addition within 15 minutes.

3. Incubate the plate at room temperature (20-24°C) for 1 hour, protected from light. Wash the plate 4 times with wash buffer. After the final wash, tap the plate firmly on absorbent paper to remove residual liquid.

4. Add 100 ul per well of TMB substrate solution (mix Reagent A and Reagent B in equal volumes immediately before use and equilibrate to room temperature). Incubate at room temperature (20–24°C) for 10 minutes in the dark. Do not shake.

5. Add 100 ul per well of stop solution. Gently tap the plate for approximately 5 seconds to ensure mixing.

6. Measure absorbance at 450 nm using a microplate reader. For research use only

## Precautions

1. If the BSA concentration in a sample is expected to exceed 32 ng/ml, dilute the sample appropriately before testing to ensure results fall within the assay range.

2. For lyophilized samples, reconstitute completely using sample diluent. Mix thoroughly until fully dissolved before analysis.

3. During incubation, keep the microplate in a clean, sealed environment to avoid contamination or evaporation.

4. After washing, ensure all residual liquid is completely removed by tapping the plate on absorbent paper. Avoid residual bubbles in wells.

5. After opening, return unused strips to the original sealed bag with desiccant and store at 4°C.

6. Use only BSA-free consumables throughout the assay. Ensure pipette tips, wash bottles, and all containers are free from BSA contamination.

## Results Interpretation

1. Assay Validity Criteria The assay results are considered valid only if all of the following criteria are met:

• Positive reference: 18–22 ng/mL $R^2$ >= 0.98 •

2. Standard Curve and Calculation Plot a standard curve in Excel using a scatter plot, with BSA concentration (ng/mL) on the x-axis and blank-corrected OD values on the y-axis. Fit the data using an appropriate regression model and display the equation and $R^2$ value. Calculate sample concentrations by substituting the blank-corrected OD values into the regression equation. For research use only Example Data

| Standard concentration (ng/ml) | OD1 | OD2 | OD3 | Mean OD | Corrected OD |
|---|---|---|---|---|---|
| 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. Interpretation Based on the standard curve, define the following OD ranges: Range A: OD corresponding to 0–16 ng/mL •

• Range B: OD corresponding to 16–32 ng/mL

• Range C: OD corresponding to >32 ng/mL 3.1 If the measured OD value is lower than the OD corresponding to 2 ng/mL on the standard curve, the BSA concentration should be reported as < 2 ng/ml. 3.2 If OD values from different dilutions of the same sample all fall within Range A, the result from the lowest dilution factor (i.e., least diluted sample) should be used for calculation. For research use only 3.3 If OD values from different dilutions all fall within Range B, or span Range A and B, or Range B and C, select the value closest to the OD corresponding to 16 ng/mL for calculation. 3.4 If OD values from different dilutions all fall within Range C, the sample should be further diluted and re-tested to obtain values within the OD range corresponding to 2-32 ng/ml. The calculation should then follow Sections 3.2-3.4 above. 3.5 If a higher dilution yields a higher OD value than a lower dilution or undiluted sample, this may indicate procedural error or excessively high BSA concentration (hook effect). The assay should be repeated with appropriate dilution adjustment. 3.6 The final result must account for the dilution factor: Actual BSA concentration = Calculated value × Dilution factor.

## Technical Specifications

1. Limit of Detection (LOD) and Limit of Quantification (LOQ) The limit of detection (LOD) is defined as the mean OD value of the zero standard plus two times the standard deviation (calculated from 20 replicates of the zero standard). For this kit, the LOD is 0.2 ng/mL (ppb). The limit of quantification (LOQ) is defined as the lowest point of the standard curve, which is 1 ng/ml.

2. Accuracy and Precision Intra-assay and inter-assay coefficients of variation (CV%) were determined at three concentration levels (20, 10, and 5 ng/mL).

• Intra-assay precision: Calculated from five replicate measurements at each concentration within the same run. Inter-assay precision: Calculated from measurements performed in three independent runs at different • times. The CV% values for both intra- and inter-assay precision are less than 10%. For research use only

| 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 |

| Inter-assay | | | | Intra-assay | | | |
|---|---|---|---|---|---|---|---|
| 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. A cross-reactivity of approximately 10% is observed with goat serum, calculated as the percentage ratio of the concentration of goat serum to BSA at the same OD value.

| Substance | Tested Concentration |
|---|---|
| Human serum albumin | 10 mg/mL |
| Mouse serum | 10% |
| Rabbit serum | 10% |
| Fish gelatin | 1% |

4 Hook Effect When the sample concentration is significantly higher than the highest point of the standard curve, a hook effect (prozone effect) may occur, resulting in OD values lower than those corresponding to 32 ng/mL BSA. For this kit, the hook effect may be observed at BSA concentrations above 500 ug/ml.