Version 01
Update: 06/29/2023

# Product Manual

# BSA ELISA Kit, 2G

## Cat. No. L00976

**For Research Use Only. Not for Use in Diagnostic Procedures.**

The operator should read technical manual carefully before using this product.

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## I. DESCRIPTION

GenScript BSA ELISA Kit, 2G (Cat. No. L00976) is a Sandwich ELISA Kit that is designed for quantitative measurement of bovine serum albumin (BSA) in testing samples. These outstanding features make it a great solution for routine BSA impurity detection, quality control and lot release, as well as purification process optimization.

Most commercial serum-free media are formulated with various biological macromolecules that can replace the function of serum, such as bovine serum albumin (BSA), transferrin, and insulin. The use of serum-free media can greatly reduce the amount of potential impurities, but the final product serving therapeutic purpose should be highly purified and quality controlled. Hence, it is necessary to test for trace media impurities, and the residual concentration of BSA is usually an important indicator for quality control in production.

## II. ASSAY PRINCIPLE

BSA ELISA Kit, 2G utilizes two anti-BSA monoclonal antibodies which bind to different epitopes of BSA. When the sample and the anti-BSA detection antibody conjugated with biotin are added to the plate (Capture Plate) coated with the anti-BSA capture antibody, the capture antibody and the detection antibody (Biotin Anti-BSA Antibody) bind to the BSA in the sample in a sandwich format. Streptavidin-Horseradish Peroxidase conjugate (Streptavidin-HRP) is added to interact with the Biotin Anti-BSA Antibody. After washing steps, 3,3',5,5'-Tetramethylbenzidine solution (TMB Solution) is added, resulting in formation of blue color. The reaction is stopped by adding Stop Solution. Application of the Stop Solution results in the color changing from blue to yellow. The intensity of the color can be read at 450 nm and 650 nm by a microplate reader. The quantity of BSA in the sample is precisely quantified against a BSA standard curve.

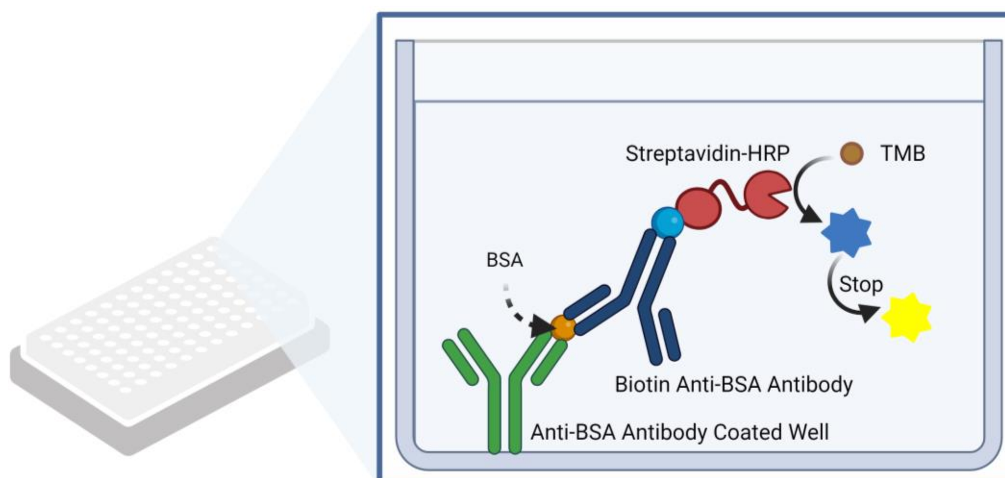


**Figure 1:** Schematic diagram of BSA ELISA Kit, 2G



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## III. KIT CONTENTS

The kit provides the following reagents and items.

| Component | Quantity/Size | Part No. |
|---|---|---|
| Capture Plate | 1 plate | J1-80 |
| Standard Stock | 1 vial (50 µL) | J1-10 |
| Biotin Anti-BSA Antibody | 1 bottle (12 mL) | J1-20 |
| Streptavidin-HRP | 1 bottle (12 mL) | E1-30 |
| Sample Dilution Buffer | 1 bottle (40 mL) | J1-60 |
| TMB Solution | 1 bottle (12 mL) | A1-40 |
| 20× Wash Solution (2% Tween-20) | 1 bottle (40 mL) | F1-70 |
| Stop Solution | 1 bottle (6 mL) | A1-50 |
| Plate Sealer | 2 pieces | N/A |

- Capture Plate: 96 well microplates (8 wells x 12 strips); 12 strips are configured in plate; plate is sealed in a foil pouch with a desiccant.
- Standard Stock contains 3.2 µg/mL of BSA. The Standard Stock shall be diluted with Sample Dilution Buffer to generate BSA standard curve.

## IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

## V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder
- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes
- 10 µL, 200 µL, and 1000 µL pipette tips
- Multichannel pipettes



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- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 37 ± 2 °C and 25 ± 2 °C incubator
- Constant temperature shaker

## VI. PRECAUTIONS

1. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials in accordance with local regulations. For more detailed information, please contact your local lab safety committee.
2. All personnel working with viral vectors must complete appropriate Biosafety Training.
3. Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
4. Avoid contact of skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep container tightly closed. In case of an accident, please seek medical assistance immediately.
5. Do not use the kit if there is any visible damage to the packaging or kit contents.
6. Do not mix components from different batches. Do not mix with components from other manufacturers.
7. Do not use reagents beyond the stated expiry date.
8. All reagents must be equilibrated to room temperature (20°C - 25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contamination may occur.
9. Before opening the Standard Stock, quick span the vial to ensure that all the reagent has collected at the bottom, and prevent the reagent from splashing when opening the lid.
10. Use only distilled or deionized water and clean glassware.
11. Do not let wells dry during the test, add reagents immediately after completing washing steps.

## VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. Sample stability has not been evaluated. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria that meet their needs.



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2. Store specimens at -20°C or lower if not testing immediately. Avoid repeated freeze-thaw cycles.

## VIII. PROTOCOL

- **Reagent Preparation**

All reagents must be equilibrated to room temperature before use (20°C to 25°C). All samples and reagents should be vortexed before use. Store all reagents back in refrigerator promptly after use.

**1× Wash Solution:** Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C when not in use.

*Note: If any precipitate is found in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.*

**Sample preparation**

If your sample is **cell culture supernatant**, we recommend several 10-fold serial dilutions for the samples to ensure that at least one diluted sample is in the range of the standard curve. Usually, 10-10,000-fold dilutions of the sample are required. It is recommended that all samples be prepared in duplicate. The results are multiplied by the dilution factor to determine the BSA concentration in the testing sample.

If your sample is **blood or serum**, please handle all the blood and serum samples as if they are capable of transmitting infectious agents. We recommend at least 1000-fold dilution of the sample to prevent cross-reactivity. Do not use hemolyzed, clotted, contaminated, or viscous specimens. Samples containing particulate matter should be centrifuged.

Store all samples at -20°C or lower if not testing immediately. Avoid repeated freeze-thaw cycles. The handling and storage information provided here is based on references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

**Standard working solution**

Dilute Standard Stock with Sample Dilution Buffer with a volume ratio of 1:100. For example, mix 10 µL of Standard Stock with 990 µL of Sample Dilution Buffer to make 1 mL of 32 ng/mL standard working solution with label of "Std-01". The recommended dilution gradient is as follows: 32, 16, 8, 4, 2, 1, 0.5, 0 ng/mL, which is used to establish standard curve.

Prepare seven 1.5 mL tubes labeled numerically from Std-02 to Std-08 consecutively, and pipette 500 µL of Sample Dilution Buffer into each tube.

Pipette 500 µl of 32 ng/mL standard solution from Std-01 tube into the Std-02 tube and mix to make 16 ng/mL of standard working solution. Then pipette 500 µL of the solution from the



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former tube into the latter one according to these steps.

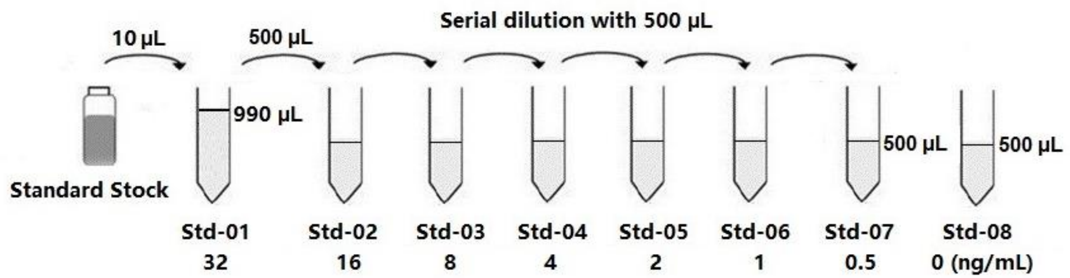


**Figure 2:** Diagram of Serial Dilution.

- **Capture Plate Preparation**

1. It is recommended that all standards be prepared at least in duplicate.
2. Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.
3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

**Table 1**. Setup of BSA standards and samples on Capture Plate

| | Standards | | Samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std-1 | Std-1 | S1 | S9 | S17 | S25 | S33 | S41 | S49 | S57 | S65 | S73 |
| B | Std-2 | Std-2 | S2 | S10 | S18 | S26 | S34 | S42 | S50 | S58 | S66 | S74 |
| C | Std-3 | Std-3 | S3 | S11 | S19 | S27 | S35 | S43 | S51 | S59 | S67 | S75 |
| D | Std-4 | Std-4 | S4 | S12 | S20 | S28 | S36 | S44 | S52 | S60 | S68 | S76 |
| E | Std-5 | Std-5 | S5 | S13 | S21 | S29 | S37 | S45 | S53 | S61 | S69 | S77 |
| F | Std-6 | Std-6 | S6 | S14 | S22 | S30 | S38 | S46 | S54 | S62 | S70 | S78 |
| G | Std-7 | Std-7 | S7 | S15 | S23 | S31 | S39 | S47 | S55 | S63 | S71 | S79 |
| H | Std-8 | Std-8 | S8 | S16 | S24 | S32 | S40 | S48 | S56 | S64 | S72 | S80 |

**Std:** Standard number; **S:** Sample number

- **Test Procedure**

**Samples and Detection Antibody Incubation**

1. Add 50 µL of standard working solutions and samples to the corresponding wells in the Capture Plate.
2. Add 100 µL of Biotin Anti-BSA Antibody to all wells in the Capture Plate.



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3. Cover the plate with Plate Sealer and incubate on rotary shaker at 300 rpm for 60 minutes, at 37°C.
4. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution four times.
5. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Enzyme Conjugate Incubation**

6. Add 100 µL of Streptavidin-HRP to all the testing wells.
7. Cover the Plate with Plate Sealer and incubate on rotary shaker at 300 rpm for 10 minutes, at 37°C.
8. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution four times.
9. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps.

**Substrate Reaction and Absorbance Measurement**

10. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
11. Add 50 µL of Stop Solution to each well to stop the reaction.
12. Read the absorbance in microplate reader at dual wavelengths of 450 nm and 650 nm immediately. Then the absorbance at 650 nm which serves as the reference is **subtracted** from the absorbance of the same well measured at 450 nm to eliminate the background signal that comes from turbidity.



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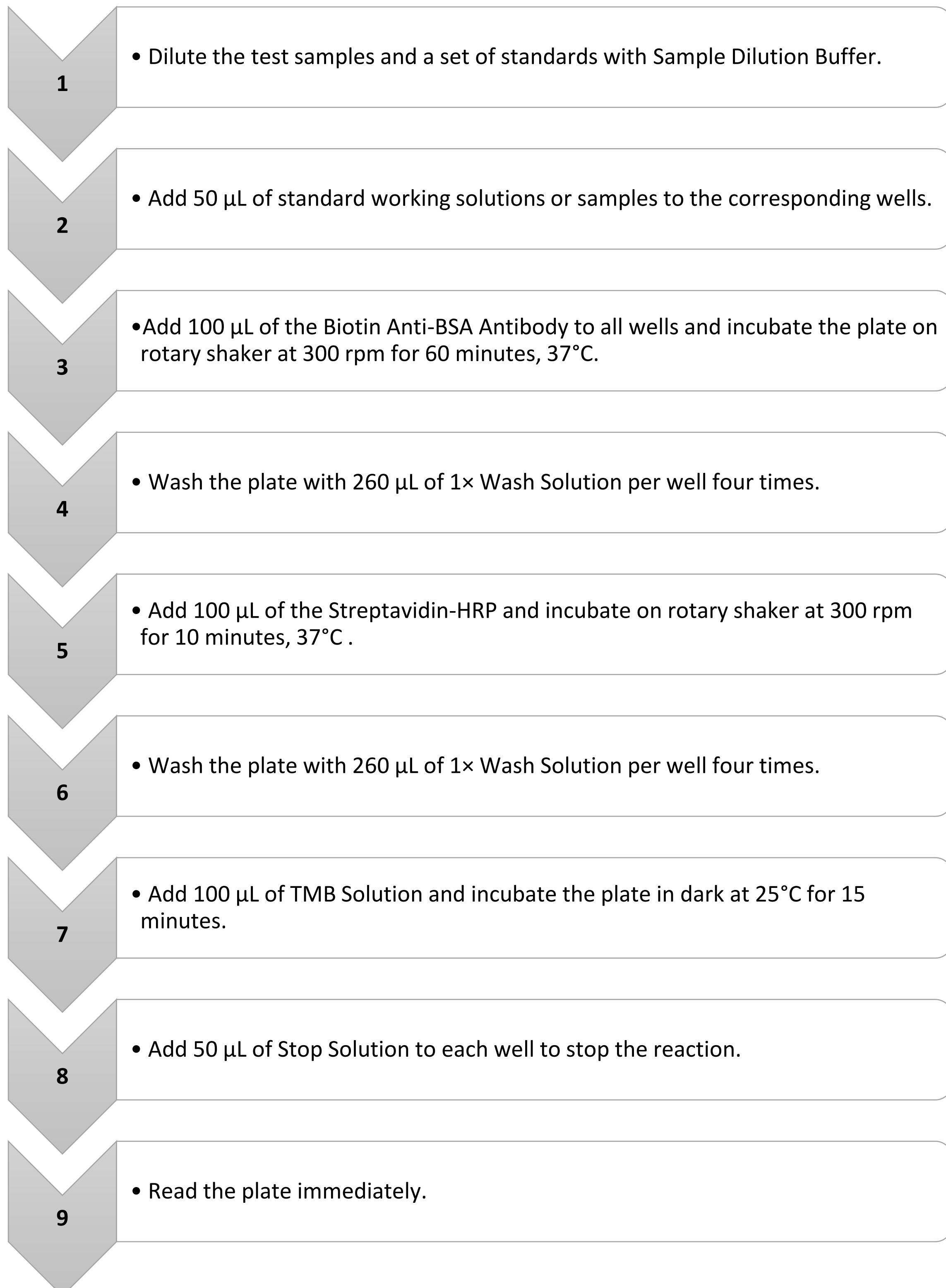
## IX. ASSAY PROCEDURE SUMMARY



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## X. INTERPRETATION OF RESULTS

- **Assay Validation**

To ensure the validity of results, the following criteria noted in the Table 2 are required. If a test fails to meet the requirements, the test is invalid and must be repeated.

**Table 2**. OD 450/650 nm values for quality control

| Items | Special Test | Requirements |
|---|---|---|
| 1 | OD 450/650 nm value for 32 ng/mL standard | > 1.5 |
| 2 | OD 450/650 nm value for 0 ng/mL standard | < 0.1 |

*Note：The standards in the table are only intended to evaluate the performance of the kit.*

- **Suggested Calculation of Data**

Statistical software can be used to create the standard curves. Choose a method with high Goodness of Fit (R^2) to analyze the data, such as a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

**Manual method for demonstration:**

The standard curve has been provided for demonstration only. It should be prepared each time an assay is performed.

1. Plot standard curve with the BSA concentration (ng/ml) on the x-axis and the corresponding mean absorbance value on the y-axis.
2. Using a four-parameter logistic curve fitting program, calculate the best fitting linear line through the points of the standard curve.

The equation for the model is:

$$y = D + \frac{A - D}{1 + (\frac{x}{C})^B}$$

A = the minimum value that can be obtained (i.e. what happens at 0 dose)

D = the maximum value that can be obtained (i.e. what happens at infinite dose)

C = the point of inflection (i.e. the point on the S shaped curve halfway between A and D)

B = Hill's slope of the curve (i.e. this is related to the steepness of the curve at point C)

3. Determine the BSA concentration (ng/mL) for the samples with the standard curve.



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**Table 3**. Sample data for standard curve.

| Standard | Absorbance (450/650 nm) | | |
|---|---|---|---|
| (ng/mL) | Duplicate 1 | Duplicate 2 | Average |
| 32 | 2.3550 | 2.5012 | 2.4281 |
| 16 | 1.2200 | 1.2195 | 1.2198 |
| 8 | 0.6999 | 0.6900 | 0.6950 |
| 4 | 0.3401 | 0.3825 | 0.3613 |
| 2 | 0.1745 | 0.1830 | 0.1788 |
| 1 | 0.1036 | 0.1037 | 0.1037 |
| 0.5 | 0.0639 | 0.0644 | 0.0642 |
| 0 | 0.0276 | 0.0275 | 0.0276 |

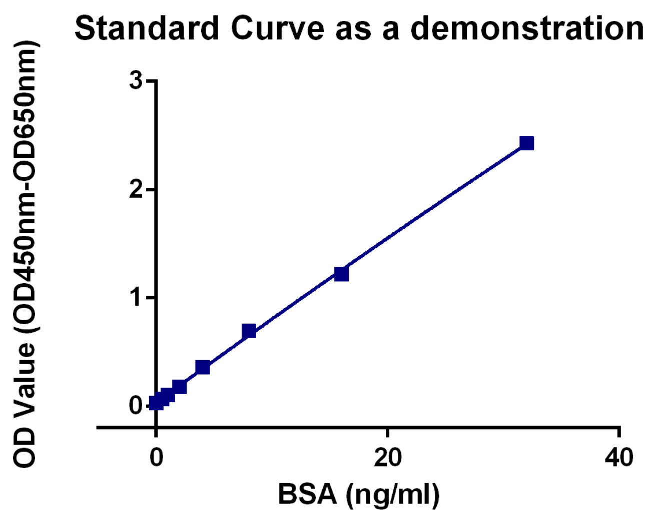


**Figure 3:** BSA ELISA Kit, 2G standard curve.

## XI. ANALYTICAL PERFORMANCE

- **Linear Range**

The linear range of this kit is from 0.5 to 35 ng/mL according to the evaluation method recommended in CLSI guideline EP06-A [2].

- **Detection Capability**

The LoB (Limit of Blank) of this kit is 0.14 ng/mL.

The LoQ (Limit of Quantitation) of this kit is 0.5 ng/mL.

The LoB is defined as that concentration corresponding to a signal two standard deviations above the mean of the zero standard. The LoQ is defined as the lowest concentration, where concentration coefficients of variation (CVs) are less than 20%. This evaluation method is

recommended in CLSI guideline EP17-A2.

- **Intra-assay and Inter-assay Precision**

Intra- and inter-assay precision were evaluated by measuring the samples with 3 different concentrations.

**Table 4:** Intra-assay CV evaluation with BSA ELISA Kit, 2G

| Repeats | Average Conc. ng/mL | % CV |
|---|---|---|
| 10 | 2.0 | 5% |
| 10 | 14.4 | 5% |
| 10 | 21.5 | 5% |

**Table 5:** Inter-assay CV evaluation with three-batch BSA ELISA Kit, 2G

| Inter-assay | | | |
|---|---|---|---|
| **Batch Amount** | **Repeats** | **Average Conc. ng/mL** | **% CV** |
| 3 | 3×10 | 2.1 | 7% |
| 3 | 3×10 | 14.3 | 9% |
| 3 | 3×10 | 22.1 | 8% |

- **Recovery/Interference Studies**

In recovery experiment, a known amount of BSA is added to the buffer matrix, and then the BSA ELISA Kit, 2G is used to measure the recovery (concentration) of the BSA-buffer matrix. Extreme pH, high salt concentrations, certain high protein concentrations, and certain detergents may result in inadequate recovery. The user can dilute the standards provided in this kit with the sample matrix of interest and perform recovery experiments to identify whether the sample can be accurately recovered in the sample matrix. This is necessary to determine the accuracy of the experiment.

**Table 6:** Recovery rate of BSA ELISA Kit, 2G in duplicate

| Repeats | Average Conc. ng/mL | Recovery Rate |
|---|---|---|
| 2 | 2 | 107% |
| 2 | 3.2 | 114% |
| 2 | 7.8 | 101% |
| 2 | 13.5 | 105% |
| 2 | 25 | 95% |

- **Hook Effect**

When the BSA concentration is greater than 32 ng/mL, the testing concentration of the BSA is inaccurate. The hook effect occurs when the concentration of BSA in the testing sample reaches 30 μg/ml, which means that the absorbance value when testing BSA at concentrations greater than 30 μg/ml will be less than the value of the standard at 32 ng/mL.



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## XII. TROUBLESHOOTING

| Problem | Probable Cause | Solution |
|---|---|---|
| **Poor Precision** | Wells are not washed or aspirated properly | Make sure the wash apparatus works properly and wells are dry after aspiration |
| | Wells are scratched with pipette tip or washing needles | Dispense and aspirate solution into and out of wells with caution |
| | Particulates are found in the samples | Remove any particulates by centrifugation prior to the assay |
| **Weak/No Signal** | Substrate is not added or added at the wrong time | Follow the manual to add the substrate properly |
| | Components are used from other lots or sources | Use only lot-specific components |
| | Substrate is contaminated | Use new Substrate from the same Lot |
| | Volumes of reagents are not correct | Repeat assay with the required volumes as noted in the manual |
| | The plate is not incubated for proper time or temperature | Follow the manual to repeat the assay |
| | The plate is not read within the specified time range | Read the plate within 5 minutes |
| **High Background** | The plate is not washed properly | Make sure the wash apparatus works properly |
| | Substrate is contaminated | Use new substrate from the same Lot |
| | Evaporation of wells during incubations | Perform incubation steps with a plate sealer in a repeat assay |
| | Incorrect incubation times and/or temperatures | Follow the manual to repeat the assay |



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## XIII. REFERENCES


1. Francis G L. Albumin and mammalian cell culture: implications for biotechnology applications [J]. *Cytotechnology*, 2010, 62(1): 1-16.
2. CLSI document EP06-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2nd Edition).
3. CLSI document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition).


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