Bovine Serum Albumin Capture ELISA

Product Insert

Photometric Format

Cat. No. AK249-2 (2 x 96 wells)

For Research Use Only

Introduction

The advent of biological products produced using animal components has created a need to ensure their removal prior to use. This removal can be accomplished in a number of ways but in all cases, must be verified by the use of a sensitive and specific assay methodology.

Fetal calf serum is commonly used in tissue culture processes in concentrations of 1 – 10%. Guidelines published by a 1987 WHO study group (WHO Tech. Rep. Ser., 747, 1987) suggest a residual concentration of no more than 1 part per million in the final product (1 µg/ml).

Since fetal calf serum is largely Bovine Serum Albumin, this protein was chosen as the basis of the current assay.

The assay can be completed in less than 2 hr. The test kit incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from complex sample matrix effects and/or the nonspecific adsorption of reactive test components, resulting in improvements in both sensitivity and specificity. Two- and five-plate test kits are available in a standard photometric detection format (AK249-2 or AK249-5).

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Components

1. BSA Antigen Capture Plate (96 tests)-2 ea.
2. BSA Standard (100x, 2,500 ng/ml)-200 ul
3. BSA Detection Antibody, HRP-labeled (1x) - 22 ml
4. Wash Buffer (20x)-30 ml
5. Chromagen Solution (1x)-22 ml
6. Stop Solution (1x)-22 ml
7. BSA ELISA Buffer (1x)-30 ml
8. Sample Dilution Tray-2 ea.

Storage

Store all kit components at 2-8° C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

Procedure

1. Remove the kit components from storage and allow the components to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample and eight wells for the generation of a standard curve. The remaining test wells and dessicant should be returned to the pouch, sealed, and stored at 2-8° C.
3. To prepare a standard curve, add 10 ul of **BSA Standard** to 1000 ul of **BSA ELISA Buffer**. This will result in a concentration of 25 ng/ml. Prepare six, additional 2-fold serial dilutions of the diluted BSA Standard to complete the standard curve by transferring 500 ul of the first dilution (25 ng/ml) to 500 ul of BSA ELISA Buffer and repeating until the appropriate number of dilutions have been prepared.
4. The **100x BSA Standard** is supplied at 2500 ng/ml, so the initial dilution plus the six additional dilutions will result in the following concentrations: 25, 12.5, 6.3, 3.1, 1.6, 0.8, and 0.4 ng/ml. **BSA ELISA Buffer** should be used as the 0 ng/ml standard.
5. To begin the assay, transfer 100 µl of sample or standard curve to the appropriate wells of the **BSA Capture Plate**.
6. Cover the plate and incubate for 45 min. at room temperature on a plate shaker set at moderate speed.
7. Wash the wells 5x with at least 300 µl/well **1x Wash Buffer**.
8. Add 100 µl of **BSA Detection Antibody** to each well, cover the plate and incubate for 45 min. on a plate shaker using the same settings (Step 6).
9. Wash the wells 5x with at least 300 µl/well **1x Wash Buffer**.
10. Add 100 µl of **Chromagen** to each well and incubate for 10 min. on a plate shaker.

11. Stop the reaction by the addition of 100 µl of **Stop Solution**.
12. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

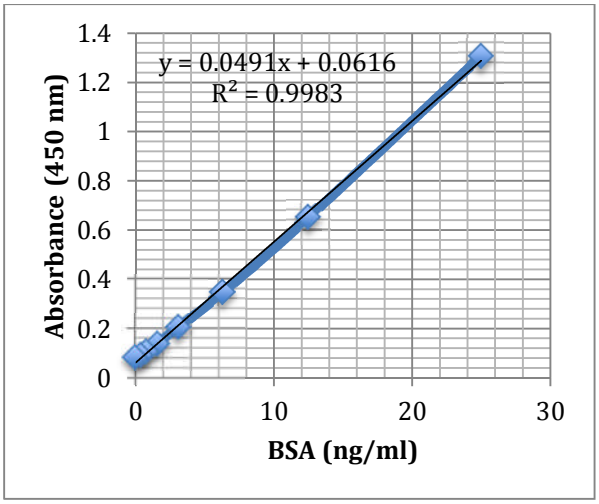
Quality Control

1. The 0 ng/ml standard absorbance value should be ≤0.250.
2. The 25 ng/ml standard absorbance value should be ≥0.500.

Standard Curve and Interpretation of Results

1. Prepare a standard curve by plotting the absorbance for each standard on the y-axis against the BSA concentration of the x-axis.
2. Determine the BSA concentration for each unknown sample by interpolation from the standard curve.
3. Samples with values ≥ 25 ng/ml may be diluted in **BSA ELISA Buffer** as described above and re-tested.

Sample Calibration Curve



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