

Human Serum Albumin

Catalog. No: KBBP07

Immunoassay for the measurement of *Human Serum Albumin*

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Introduction:

A variety of products, manufactured using the biotechnology route such as cell or tissue culture may result in residual contamination of the end-product. Trace contamination of HSA in pharmaceutical and other biological products is one such area. Such trace contaminants may lead to potential health hazards in humans, especially when the product is intended for therapeutic use.

Intended Use:

This generic kit is intended in determining the presence of Human Serum Albumin contamination in various products that are manufactured through the biotechnology route. The kit has been validated successfully for testing of final product for HSA in variety of products regardless of growth and purification process.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing Human Albumin are reacted with anti HSA-HRP antibody simultaneously in the microtiter wells already coated with affinity purified capture HSA antibody. This immunological reaction results in formation of a sandwich complex of solid phase antibody-HSA-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure (see Assay procedure section mentioned below). The substrate 3,3',5,5' Tetramethyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of HSA present.

Materials Provided:

1. Anti-HSA coated Microtiter plate (96 wells) – 1 no
2. Anti-HSA:HRP, 11mL – 1 bottle
3. HSA Standards, (0.5mL/vial) – 0, 0.5, 2, 5, and 20 ng/mL
4. TMB Substrate, 11mL – 1 bottle
5. Wash Buffer (10X), 100 mL – 1 bottle
6. Stop Solution, 11mL – 1 bottle
7. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm and 630 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 µL to 100 µL.
3. Distilled water.
4. Wash bottle or automated microplate washer.
5. Log-Log graph paper or software for data analysis.
6. Timer.
7. Absorbent paper.
8. 1 Liter bottle for diluted Wash Buffer

Handling/Storage:

1. All reagents should be stored at 2^oC to 8^oC for stability.
2. All the reagents and wash solution are stable until the expiration date of the kit.
3. The substrate reagent should not be used if its stopped absorbance at 450nm is greater than 0.2
4. Before using, bring all components to room temperature (18-25 °C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and expiration date and store it at appropriate conditions mentioned.
2. Dilute Wash Buffer to 1 Liter in Distilled water and store at 4^oC.
3. Bring all reagents to room temperature before use.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of HSA. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of HSA present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the HSA concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of samples containing sodium azide (NaN₃), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of HSA.
4. All Standards, Controls and Samples should be assayed atleast in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards, Controls and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. Pipette out 25 μ L of **Standards, Controls** and **Samples** into the respective wells as mentioned in the worklist.
3. Pipette out 100 μ L of anti-HSA:HRP **Conjugate** into each well.
4. Cover the plate and incubate it on a plate shaker at ~180rpm for 90 minutes at room temperature, $22^{\circ}\text{C}\pm 4^{\circ}$ or incubate for about 150 minutes without shaking.
5. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Pipette out 100 μ L of TMB **Substrate** in each well.
7. Incubate the plate at room temperature for 30 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color
8. Pipette out 100 μ L of **Stop Solution**. Wells should turn from blue to yellow in color.
9. Read the absorbance at 450/630nm blanking on the Zero Standard.

Example of a Work list

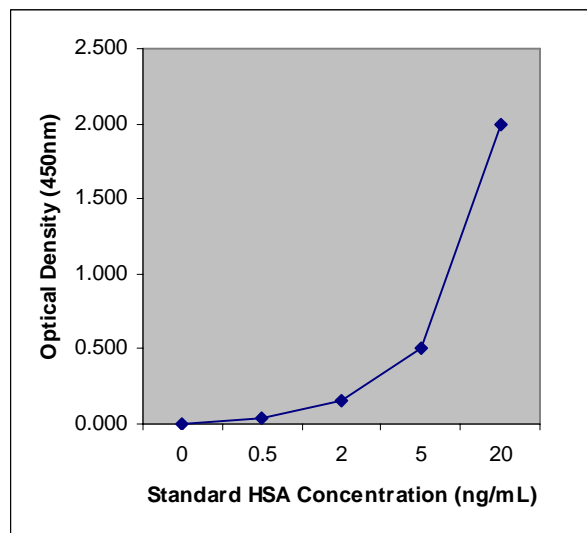
Well #	Contents	Abs at 450/630nm	Mean Absorbance	ng/ml HSA equiv.
1A	Zero Std			
1B	Zero Std			
1C	0.5 ng/mL			
1D	0.5 ng/mL			
1E	2 ng/mL			
1F	2 ng/mL			
1G	5 ng/mL			
1H	5 ng/mL			
2A	20 ng/mL			
2B	20 ng/mL			
2C	Sample A			
2D	Sample A			
2E	Sample B			
2F	Sample B			

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards, Controls and Samples. Subtract the Mean Absorbance of the Zero Standards (background) from each well. Plot the standard curve on log-log graph paper, with Standard HSA Concentration on the X-axis and Absorbance on the Y-axis. Draw the best fit straight line through the standard points. To determine the unknown HSA concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the HSA Concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.

**Precautions:**

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

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