

INTENDED USE

The Human anti-BSA Ig ELISA Kit is an immunoassay suitable for quantifying or titrating total antibody activity specific for bovine serum albumin (BSA) in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

GENERAL INFORMATION

Bovine serum albumin (BSA) is the major protein of bovine serum (also fetal calf serum), used as a required nutrient for the *in vitro* culture of many cell types, including cell lines for production of recombinant proteins used as pharmaceutical drugs. Such recombinant protein culture products are purified from culture medium components, including BSA, which even as minor contaminants may affect drug efficacy and side effects. BSA is also highly immunogenic in mammals and can, therefore, elicit anti-BSA antibodies in the host receiving the BSA-contaminated drug. The measurement of residual culture components such as BSA and the immunological host response, i.e., anti-BSA antibodies, is a requirement of the manufacturing processes of recombinant protein drug production.

Purified BSA is also used as a carrier protein for chemical attachment of small molecules, including peptides and other haptens, to produce an effective vaccine for production of antibodies specific for the attached molecule. The BSA-hapten immunization effectively produces anti-BSA antibodies concomitant with anti-hapten antibodies. These 'by-stander' antibodies, which may interfere with the subsequent use and interpretation of the anti-hapten activity, are commonly removed by solid phase BSA adsorbents.

The ADI Human Anti-BSA Ig ELISA is a sensitive and convenient method for detecting and quantifying anti-BSA IgG antibodies arising from BSA contamination of administered drugs, and for following anti-BSA antibodies from carrier-hapten immunizations.

PRINCIPLE OF THE TEST

The Human anti-BSA Ig ELISA kit is based on the binding of human anti-BSA Ig in samples to BSA immobilized on the microwells, and anti-BSA Ig antibody is detected by anti-human IgG+IgA+IgM-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-BSA present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human Ig antibody in samples is calculated relative to anti-BSA calibrators.

PRODUCT SPECIFICATIONS

Specificity

Purified BSA is used to coat the microwells; thus the assay is specific for antibodies directed to BSA. The anti-Human Ig HRP conjugate reacts specifically with human IgG, IgA and IgM antibodies that bind to BSA on the plate; IgE antibody would not be measured above background signals.

Assay Sensitivity

The BSA coating level and HRP conjugate concentration are optimized to differentiate anti-BSA Ig from background (non-antibody) signal with human serum samples diluted 1:200.

Calibrator Values

The calibrators are dilutions of antibody reactive to BSA. Values are assigned as arbitrary anti-BSA activity units (see Limits of the Assay).

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KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20B, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
Anti-Human Ig- HRP Conjugate Concentrate (100x) Part: H-HuGAM.112, 0.15ml	Peroxidase conjugated anti-Human Ig in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
BSA Microwell Strip Plate	8001	8-well strips (12)	Coated with BSA, and post-coated with stabilizers.
Anti-BSA Calibrators			
10 U/ml	710-132B	0.65 ml	Four (4) vials, each containing anti-BSA IgG levels in arbitrary activity Units; diluted in buffer with detergents and antimicrobial as stabilizers.
25 U/ml	710-132C	0.65 ml	
50 U/ml	710-132D	0.65 ml	
100 U/ml	710-132E	0.65 ml	
Human Anti-BSA Positive Control	710-142	0.65 ml	Human serum with anti-BSA activity; diluted in buffer with protein, detergents and antimicrobial as stabilizers
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human Ig HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

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PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

LIMITATIONS OF THE ASSAY

Quantitation of Antibody in a Sample

The ELISA measures anti-BSA activity, a combination of antibody concentration and avidity for the BSA antigens. Antibodies with substantially different total Ig concentrations may display similar anti-BSA activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of Ig (e.g., ug/ml).

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in BSA avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 25 U/ml Calibrator, or another Calibrator in the kit (see Calculation of Results).

ASSAY DESIGN AND SET-UP

CAUTION: Avoid solutions or containers with BSA, which would block or diminish anti-BSA activity in samples.

Sample Collection and Handling

Culture medium (BSA-free), serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

Assay Design

Review Calculation of Results (p5-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be <0.5 OD. This is usually 1/200 or greater dilution for human sera with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required (**See Method A,B**). Blank OD should be <0.3.
- Run a set of **Calibrators**. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, **Method C**, has limitations. See Limits of the Assay (above).
- Run the Human anti-BSA **Positive Control**. A net OD value greater than **0.5** indicates proper assay performance.
- Run a range of sample dilutions for expected higher positives that allows calculation of antibody **Titer** (when specific titer is at least 4-fold higher than non-immune). **See Method D**.
- Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

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Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human Ig HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

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INTERPRETATION OF RESULTS

Calculation of Results

Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine **Positive Immune** and **Negative Non-immune**, and to **Quantitate** positive antibody levels.

Method A. Antibody Activity [ELISA Signal & Sample Dilution]

Represent data as net OD units (A450 signal; blank subtracted) ÷ dilution = **Total Activity Units**.

A Calibrator value in the mid-OD range (e.g., 25 U/ml) can be used to normalize inter-assay values.

Method B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution. This calculation **quantifies** the positive Antibody Activity level.

Example:

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.243	2.358	0.49	4.79
2	0.351	0.597	0.71	1.21
3	0.286	1.421	0.58	2.89
4	0.357	1.268	0.73	2.58
5	0.512	0.857	1.04	1.74
6	0.342	1.296	0.70	2.63
7	0.298	0.608	0.61	1.24
8	0.285	0.369	0.58	0.75
9	0.157	0.864	0.32	1.76
10	0.187	0.543	0.38	1.10
Mean	0.302			
SD	0.095			
Mean +2 SD	0.492			= Positive Index

CALCULATION OF RESULTS (continued)

Method C. Use of a Calibrator Curve

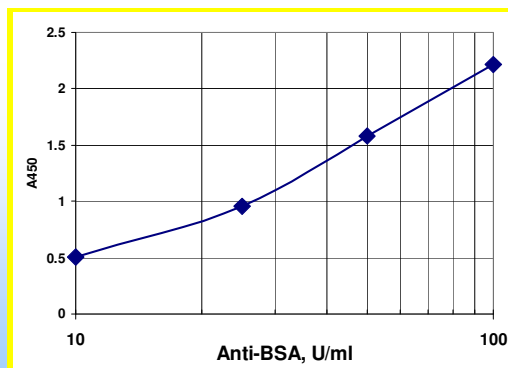
When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay), the anti-BSA activity units may be determined by interpolation from the Calibrator curve. The results may be calculated using any immunoassay software package. If software is not available, anti-BSA activity concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-BSA (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
3. The anti-BSA activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 100 U/ml calibrator should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators	A450 nm
A1,2	Negative Diluent Blank	0.16
B1,2	10 U/ml Calibrator	0.50
C1,2	25 U/ml Calibrator	0.96
D1,2	50 U/ml Calibrator	1.58
E1,2	100 U/ml Calibrator	2.21
F1,2	Sample 1:100	1.21

Sample Result: 33 U/ml x 100 dilution = **3300 U/ml**



CALCULATION OF RESULTS (continued)

Method D. Titers from Sample Dilution Curves

The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

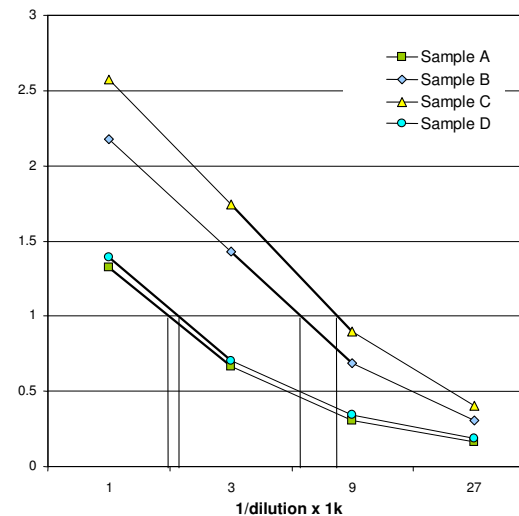
1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. A Calibrator value in the mid-OD range (e.g., 25 U/ml) can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
= **Total Ig Antibody Activity Units**

Example:

II. A 1.0 OD Index was used to determine titer of 4 antibodies.



Titer Values

Sample A = 1.72 kU
Sample B = 5.70 kU
Sample C = 1.85 kU
Sample D = 7.90 kU

Instruction Manual No. M-710-140-BSM

Human Anti-Bovine Serum Albumin (BSA) Total Ig

ELISA Kit Cat. No. 710-140-BSM

For Quantitation of anti-BSA (IgG+IgA+IgM) in Serum



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ELISA Kit Components

ELISA Kit Components	Amount	Part
BSA Coated Microwell Strip Plate	8-well strips (12)	8001
Anti-BSA IgG Calibrator	10 U/ml 0.65 ml	710-132B
Anti-BSA IgG Calibrator	25 U/ml 0.65 ml	710-132C
Anti-BSA IgG Calibrator	50 U/ml 0.65 ml	710-132D
Anti-BSA IgG Calibrator	100 U/ml 0.65 ml	710-132E
Anti-Human Ig HRP Conjugate (100X)	0.15 ml	H-HuGAM-112
Sample Diluent (20x)	10 ml	SD20B
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-710-140-BSM