Bovine Serum Albumin (BSA) Quantification with ELISA

Abstract: Bovine serum albumin (BSA) is a large, globular protein that has many biochemical functions in diagnostic, cell culture, pharmaceutical and microbiological applications. Quantifying the concentration of BSA in these various applications is essential for safety and efficacy. Bethyl Laboratories, Inc. manufactures a highly sensitive enzyme-linked immunosorbent assay (ELISA) that can detect and quantify BSA in various sample types, including serum, milk, plasma, colostrum, and cell culture. Figure 1 below shows a typical standard curve for this sandwich ELISA and our kit has an assay range of 0.69 – 500 ng/ml. This kit comes pre-coated with anti-BSA antibodies that have been validated on site, pre-blocked to avoid background noise, and with the capacity to run a total of 40 samples in duplicate.

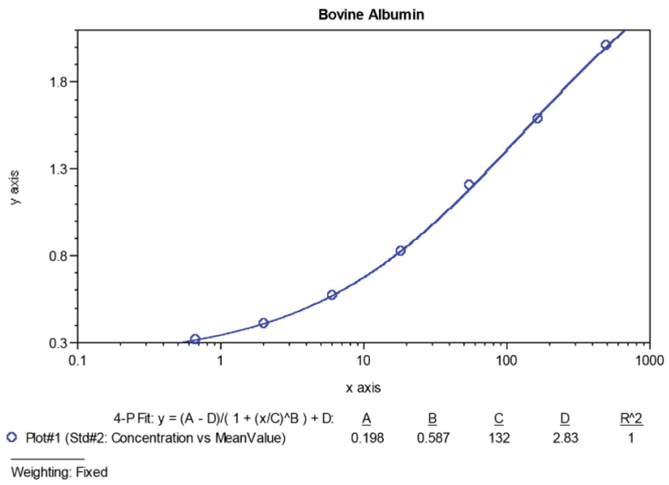

Figure 1. Bovine Albumin ELISA Kit Typical Standard Curve

This typical standard curve was generated using the Bovine Albumin ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay. Suggested standard curve points are 500 ng/ml, 167 ng/ml, 55.6 ng/ml, 18.5 ng/ml, 6.17 ng/ml, 2.06 ng/ml, 0.69 ng/ml, and 0 ng/ml.

Bovine serum albumin, also known as 'fraction V', is a physiologically important plasma protein derived from cattle. It is a monomeric protein with a single carbohydrate-free polypeptide chain consisting of 583 amino acid residues, and has a molecule weight of approximately 66,400 Da.[1] BSA is very stable, allowing it to withstand high pressures, which is likely a result of its extremely rigid structure established by 17 intramolecular disulfide bonds[2] and the presence of homologous domains (I,II,III). The main biological functions of BSA in vivo are to regulate the colloidal osmotic pressure and pH of bovine blood, as well as to transport a variety of endogenous and exogenous compounds – such as fatty acids, amino acids, hormones and drugs – around the circulatory system.[3]

The role of BSA in biotechnology

BSA is stable, moderately non-reactive and readily available from bovine blood – a byproduct of commercial meat production – thanks to the development of large-scale purification methods. In biotechnology, BSA is used in a wide variety of applications, including as a protein concentration standard,[4] a nutrient supplement in cell culture m edia, and as an additive to stabilize small molecules, enzymes and ions.

Fetal bovine serum for cell culture media

BSA is a major constituent of fetal bovine serum (FBS), the liquid fraction of clotted blood taken from fetal calves that contains nutritional and macromolecular factors essential for cell growth. It is primarily used as a protein serum supplement for in vitro eukaryotic cell culture, providing vital nutrients and growth factors for the maturation of cells.[5] Fetal serum is preferred over adult serum due to its high concentration of embryonic nutrients, lower gamma globulin content – such as antibodies that may interfere with cell binding – and the presence of fewer complement proteins that may cause cell lysis.

Universal blocking agent
BSA is used as a universal blocking agent in ELISAs, immunoblots (such as the western blot) and immunohistochemistry assays to reduce the nonspecific binding of antibodies in the sample.[6] It does this by saturating the unoccupied binding sites to minimize the binding of other proteins that are not the target molecule, which could result in high background noise and decreased assay sensitivity.

Stabilizing component
BSA is also used as a stabilizing component for protein-based reagent reactions, due to its propensity to bind, sequester, and stabilize enzymes of interest in solution, without interacting with or affecting the functions of other proteins or molecules. BSA can act as a stabilizing agent in PCR reactions that amplify poorly, by increasing the stability of DNA polymerase, and has also been shown to reverse the inhibitory effects of melanin on RT-PCR.[7] In addition, BSA can also limit the loss of reagent enzymes, including DNA polymerase, by preventing adhesion to the surface of microplate wells, tube walls or tip surfaces in immunoassays and PCR reactions, enhancing yields. Despite its widespread usage, it is still not completely known how BSA stabilizes proteins, but results suggest that the interaction between the two molecules is hydrophobic, as surface hydrophobicity has been shown to determine stabilization.[8]

ELISA quantification of BSA

ELISA is a powerful, plate-based technique used to detect and quantify the presence of target molecules – such as proteins, antibodies, and hormones – from a complex mixture. In an ELISA, the target antigen is typically immobilized on the solid surface of a multi-well microplate either directly or via the use of a capture antibody. The antigen is then detected by an antibody conjugated with reporter molecule such as an enzyme. There are four types of ELISA distinguishable by their binding patterns: direct, indirect, competitive, and sandwich (the most commonly used ELISA). For a sandwich ELISA, the immobilized primary coating antibody captures the antigen from the liquid sample, anchoring it to the well surface, then a biotinylated and enzyme-conjugated antibody specifically binds to a different epitope on the surface of the same antigen. The reporter enzyme – which is often horse radish peroxidase (HRP) for colorimetric ELISAs – then catalyzes the reaction of a colorless substrate (e.g. 3,3′,5,5′-Tetramethylbenzidine or TMB) to a colored product, which can be measured using spectrophotometry.

Veterinary testing and research
Quantifying BSA levels in the blood or milk of cattle is often used to assess their health, especially in the case of acute or chronic toxicity, or during evaluation of the efficacy of anti-infection drug treatments. Serum albumin, normally only present in bovine blood, increases in milk during mastitis, because of a loss of integrity of the blood-milk barrier caused by disruption of tight junctions between mammary epithelial cells, allowing paracellular leakage.[9] Measuring milk concentrations of BSA therefore provides a good indicator of mastitis infection severity. One team of investigators used this approach as a means to test therapeutic response to pegylated granulocyte colony-stimulating factor (PEG-gCSF) therapy for the treatment of mastitis.[10] PEG-gCSF therapy is designed to stimulate the production of granulocytes and stem cells by bone marrow to fight infection, and cows treated with PEG-gCSF had significantly reduced levels of BSA at the peak of infection. Another study used a BSA ELISA kit to understand the effect of intramammary administration of the glucocorticoid prednisolone – a treatment commonly added to antimicrobial infusions to treat mastitis – on the blood-milk barrier.[11] Prednisolone did not affect the localized immune response to mastitis, but did help to maintain the integrity of the blood-milk barrier.

Food and medicine safety
Human exposure to BSA is very common through our diet, and plays a large role in the cause of milk and beef allergies[14], as well as being linked to early-childhood membranous nephropathy[15] – damage to blood vessels in the kidneys – so it is a major concern for the food industry. BSA has been shown to cause many health complications such as allergic reactions when injected into the blood stream, which is thought to be linked to hypersensitivities to bovine gelatin.[12] A severe anaphylactic reaction was also reported in a woman that was artificially inseminated with sperm that had been processed in media containing BSA.[13] To limit BSA exposure, the World Health Organization (WHO) has set a specific guideline of 50 ng or less residual BSA per human vaccine dose, and stated that each vaccine manufacturer is responsible for demonstrating that the content of residuals is at a safe and acceptable level.

ELISA is a universally accepted method for the quantification of BSA. Sandwich ELISA-based kits are used by vaccine and biopharmaceutical manufacturers and the food industry to detect and quantify levels of BSA before releasing their products for consumer administration or consumption.

Bethyl Laboratories Bovine Albumin ELISA

Bethyl Laboratories, Inc. manufacturers a high-performance sandwich ELISA kit (Catalog No. E11-113) for the detection of bovine albumin in serum, plasma, milk, colostrum and cell culture supernatant. Each kit comes with plates that are pre-coated with an anti-BSA antibody, pre-blocked and ready to use in strips of 8 tests, with enough material to perform 40 samples in duplicate. Bethyl's team manufactures and rigorously certifies all its antibodies in-house to ensure the highest quality and sensitivity.[16]

Assay principle and protocol

The assay protocol begins with a binding step, where the BSA is immobilized/captured onto microplate wells precoated with capture antibody. The remainder of unbound proteins and molecules are then removed with a wash step. A biotinylated anti-BSA antibody is then added to the wells, which binds to the captured albumin. In contrast to common sandwich ELISA protocols, where the reporter enzyme is conjugated directly to the detection antibody, the Bethyl assay uses a separate, streptavidin-tagged horseradish peroxidase (SA-HRP) reporter to improve the limit of detection and sensitivity of the immunoassay, based on the high affinity between biotin and streptavidin.17 SA-HRP catalyzes the transfer of two electrons from the chromogenic substrate TMB to hydrogen peroxide, creating a colorimetric reaction which will turn the solution blue if BSA is present. Dilute sulfuric acid acts as a stop solution to terminate the reaction, turning the solution yellow. The absorbance of the yellow color at 450 nm (A450) is proportional to the concentration of albumin in the sample.

BSA quantification using the Bethyl Bovine Albumin ELISA kit requires the generation of a standard from purified BSA standard, with a recommended range from 0.69 to 500 ng/ml. The diluted albumin concentrations in the test samples can then be quantified by interpolating the A450 results with the standard curve. Factoring in sample dilutions will give the concentration of albumin analyte in the original sample. Please refer to the E11-113 datasheet for detailed protocol instructions.

Assay performance

Sandwich ELISA is a very sensitive technique, and any non-specific detection of analytes can result in false positives or high noise-to-signal ratio. E11-113 kit is designed for BSA quantification only, owing to the quality of highly specific antibodies used. Bethyl's extensive testing has confirmed that E11-113 is specific for bovine albumin and not bovine IgG, IgA, IgM, bovine transferrin, or human albumin (Figure 2).

Bethyl produces its ELISA kits in house, which allows the company to have full control of the antibody life cycle. This is one of the reasons Bethyl's ELISA kits have the high level of reproducibility seen with E11-113. The E11-113 kit has been tested on a variety of samples by various researchers. We highly recommend reviewing the publication citations to see how other scientists used this kit to quantify BSA, and what kind of data they generated in their samples of interest. [10,18-20]

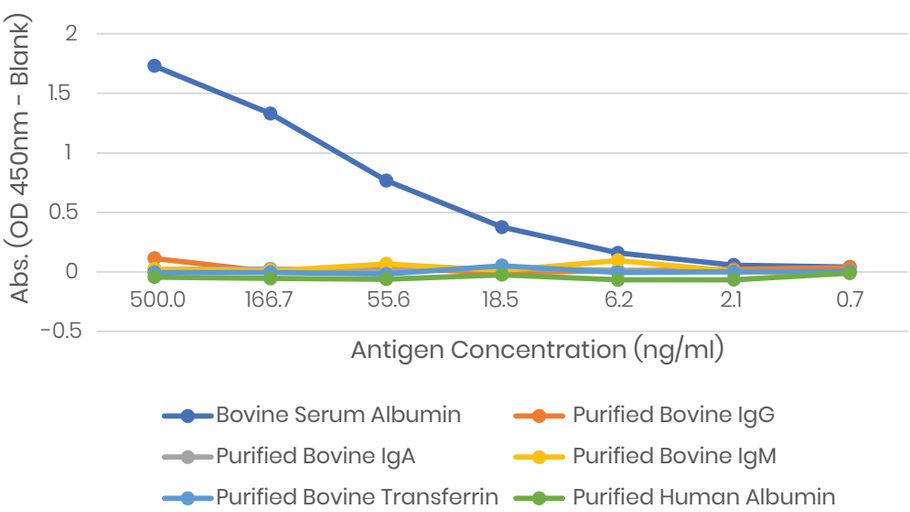

Figure 2. Specificity of BSA ELISA Kit E11-113

The antibodies used in the E11-113 ELISA kit react specifically with bovine albumin, and not bovine IgG, IgA, IgM, bovine transferrin, or human albumin. Cross-reactivity with other species has not been tested.

Tips for using E11-113

The antibodies used in this kit are highly sensitive, so off-target or background binding from workplace BSA contamination can be a problem if the environment is not well-maintained and cleaned. All equipment should be washed to avoid contamination, and Bethyl Laboratories advises that a plate washer is not used for this kit unless it is regularly cleaned and decontaminated after using other ELISA kits. In addition, these guidelines can be used to avoid contamination:

- Use a 'squirt a dump' method with a squirt bottle full of wash buffer. To do this, hold your plate at an angle upside down over the sink, and squirt the buffer into the wells, allowing it to then fall into the sink without cross-contaminating other wells. If possible, use a new squirt bottle for this purpose.
- Wipe down all surfaces, including the pipettes and benches, with diluted ethanol prior to running any ELISA in which contamination is a concern.

- If possible, prepare wash buffer and dilution buffer in new, sterile containers that are dedicated to only BSA assays.
- Do not reuse reagent reservoirs.
- Make sure that you're allowing your standard to be completely reconstituted, and use it within 24 hours of preparation.
- Perform the assay in a sterile hood to ensure that contaminants are not being introduced from the surrounding environment.

Conclusions

Bovine serum albumin is an important protein that has many functions in biotechnology, including as a universal blocking agent, a stabilizing molecule for PCR and a nutrient supplement in cell culture. Additionally, there are many instances where the concentration of BSA needs to be evaluated or controlled, such as in veterinary drug development and vaccine manufacturing. The Bovine Albumin ELISA (E11-113) kit from Bethyl Laboratories provides a sensitive method using high quality, validated antibodies for detecting and quantifying BSA concentrations in various matrices, with applications across various industries.

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BOVINE ELISA KITS
Bovine Albumin ELISA Kit, E11-113
Bovine IgA ELISA Kit, E11-131
Bovine IgG ELISA Kit, E11-118
Bovine IgG1 ELISA Kit, E11-116
Bovine IgG2 ELISA Kit, E11-117
Bovine IgM ELISA Kit, E11-101
Bovine Lactoferrin ELISA Kit, E11-126

SELECT ACCESSORIES FOR ELISA
ELISA Starter Accessory Kit, E101 (contains BSA blocking buffer)
ELISA Starter Accessory Kit II, E103 (contains non-BSA blocking buffer)
TMB One Component Substrate, E102
ELISA Blocking Buffer, E104
ELISA Wash Solution, E106

ALBUMIN ELISA KITS
Bovine Albumin ELISA Kit, E11-113
Human Albumin ELISA Kit, E88-129
Mouse Albumin ELISA Kit, E99-134
Pig Albumin ELISA Kit, E101-110
Rat Albumin ELISA Kit, E111-125

ANTIBODIES FOR ALBUMIN
Rabbit anti-Bovine Albumin Antibody, A10-127A
Sheep anti-Bovine Albumin Cross-Adsorbed Antibody A10-213A
Goat anti-Human Albumin Cross-Adsorbed Antibody A80-229A
Sheep anti-Rat Albumin Antibody A110-134A

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