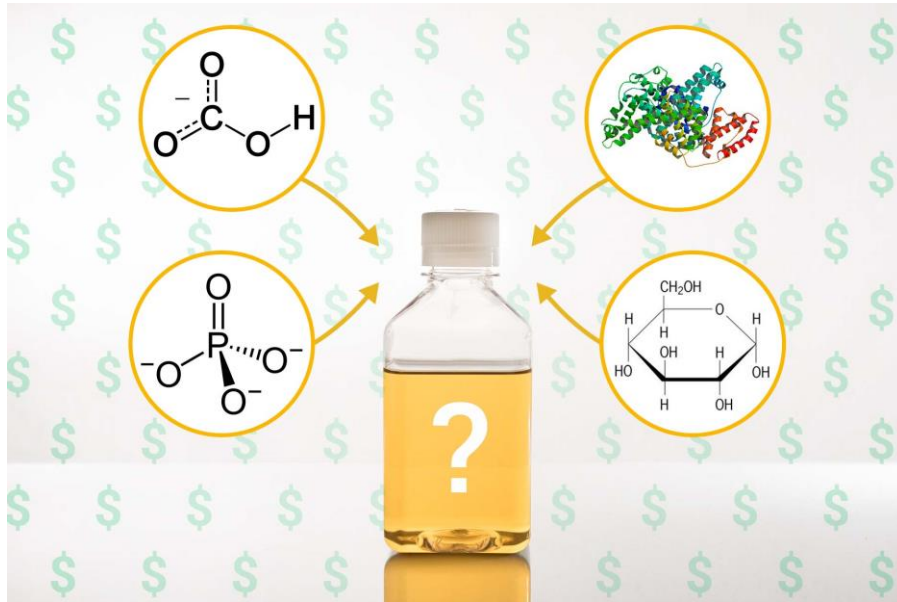


# Are You Getting What You Paid For?

## Methods for Determining Authenticity of Fetal Bovine Serum

By [Michelle Cheever](#) and [Alyssa Master, PhD](#)



We recently encountered a customer who had purchased their fetal bovine serum (FBS) at a very low price. Anytime we hear about FBS selling near or below the cost of the raw source material, we get a little curious. When we inquired about the IgG level that was listed on the product certificate of analysis, the customer asked why. We told them that it was quite possible that they had not received FBS but instead a product that included newborn calf serum (NBCS). We explained that NBCS is significantly cheaper than FBS and contains different protein concentrations that can negatively impact certain cell culture systems. If this was the case, not only did this customer overpay for their FBS by many thousands of dollars, but they also potentially confounded their research. We believe it is time to educate FBS customers. We wanted to explain the differences between FBS and NBCS and provide two simple tests that can be used to distinguish the two products.

All animal serum is complex and is comprised of water, protein, salts, sugar, amino acids, vitamins, enzymes, growth factors, and other components. All components will vary based on the age of the animal at collection, breed, sex, different geographic origin, feed, water, seasonal climatic variation, and many other factors that are yet to be determined. This is the reason that every lot is tested for each customer's suitability.

FBS is defined as the liquid fraction of naturally clotted blood that is depleted of cell, fibrin, and clotting factors derived from normal fetuses (not delivered by the birth process) from healthy dams deemed fit for human consumption.<sup>1</sup> It is collected in government inspected and registered slaughter houses. FBS is the most commonly used serum type, and is used as a supplement to provide a nutrient rich environment for *in vitro* cell growth and

maintenance. FBS is sometimes known as fetal calf serum (FCS) but should never be mistaken for calf serum. NBCS is defined as naturally clotted blood from live animals collected from birth to 20 days of age that have been deemed fit for human consumption. Bovine Calf Serum (BCS) is derived from animals collected from 20 days to 12 months of age that have been deemed fit for human consumption. All these types of serums are commercially available and can serve a specific purpose.

The use of serum has grown considerably during the past decade. Because of its high demand, a strong market and other forces limiting availability, the price of FBS will continue to rise dramatically. FBS is a multimillion dollar global industry and has historically been under-regulated. This has provided conditions that in the past have allowed for and potentially can lead to intentional deception or mischaracterization. When a customer is evaluating FBS to use for its cell lines, one must take into account many factors including geographical origin of material, the product specifications, as well as the potential for adulteration.

Manufacturing processes, documentation, and traceability have all improved over time, leading to greater control of FBS. Manufacturers of FBS should be willing participants to audits and follow industry guidelines. Supplier transparency should include active participation in quality systems and traceability programs. [International Serum Industry Association](#) (ISIA) was formed to promote and provide guidelines on compliance for standards and ethics in the business practices of global animal derived products including bovine serum. ISIA traceability certified facilities have been audited for and comply with traceability requirements.

The difference in collection and sourcing results in the cost of NBCS being about one fifth the cost of FBS. This wide cost differential has sometimes led to an issue of mislabeling and, in some instances, bargain basement pricing of some products labelled 'FBS' which in fact are 100% NBCS or some mixture of both products. *In other words, if you got an amazing price on your FBS it may be because it is wasn't 100% FBS.* Because of the biochemical differences between NBCS and FBS this could lead to lack of reproducibility or worse, incorrect results.

Some scientists might say, "hey, I don't really care what it is, it's cheap serum, I tested it, and it worked for me. what's the big deal?" We understand that budgets can be restrained which puts pressure on organizations to find ways to be more cost efficient. However, is buying cheap serum really saving you money? Is the cost of replicating an experiment due to [lack of consistent data](#) worth the savings? Is the potential reputational impact of missed timelines and/or failed experiments worth the savings? Some companies may say yes; but we suspect many will say no. In an effort to inform and educate end users, the serum industry has identified two biochemical markers that will distinguish FBS from NBCS. At this year's ISIA Annual Meeting, data was shared that definitively shows that two biomarkers, Immunoglobulin G (IgG) and Gamma-Glutamyltransferase (GGT) can be used to determine if a serum product is FBS, NBCS, or something in-between.

Serum samples were obtained from ISIA traceability certified suppliers **in association with the [ISIA](#)** to a third party to be blinded and aliquoted or directly submitted for analysis.

Biochemical properties were obtained from individual fetuses as well as pooled lots of filtered and unfiltered serum.

## The Biochemical Fingerprint of Fetal Bovine Serum (FBS)

### ***Immunoglobulin G (IgG)***

IgG is the most common antibody found in the blood and is present both in FBS and NBCS, though significantly higher levels are seen in NBCS. Varying levels of IgG in media can be incredibly problematic for anyone working in antibody production and purification. All healthy calves are born agammaglobulinemic because, in cows, the placental barrier prevents the transmission of IgG from the dam (mother) to the fetus. This antibody is largely acquired via colostrum from the maternal mammary gland causing the IgG levels of the calf to increase dramatically in the first 24-36 hours post-partum. The levels in the blood can determine if the serum is of fetal or calf origin. Although an individual fetus will have IgG levels no higher than 3000 µg/mL, due to batch processing methods the **final levels of IgG in FBS have an expected overall value of less than 300 µg/mL**. This difference in IgG presents a clear marker to demarcate between fetal and calf serum. It is important to note that the end user should be cautious when comparing unit values of IgG in certificate of analyses. It can often appear that the values are within normal range but the units are expressed differently. For example, 1000 µg/mL can be also expressed as 100 mg/dL.

### ***Gamma-Glutamyltransferase (GGT)***

GGT, sometimes called gamma-glutamyltranspeptidase, is a transferase enzyme found in the cell membranes of tissues throughout the body. This enzyme is important for the transmembrane movement of amino acids as well as the metabolism of molecules such as glutathione. Inconsistent levels of GGT can have a profound effect on the reproducibility of cell-based assays, particularly with *in vitro* cancer models due its key role in redox regulation. The serum levels of this enzyme are vastly different pre- and post-partum because, like IgG, GGT is found in colostrum. In mammals, the GGT levels in fetal origin serum change as the gestation period progresses, but remains lower when compared to calf origin blood. This enzyme serves as a distinct biomarker for determining if serum is fetal or calf due to the disparate differences in the levels seen within a fetus (~0-5 IU/L) and those seen in calves (~160-1000 IU/L). Variations are expected to be seen based on country of origin and type of analyzer used. However, typical GGT levels for FBS in US origin product usually fall below 5 IU/L while that for Australia/NZ usually fall below 12 IU/L.<sup>2</sup>

### ***#FakeFBS***

With experimental reproducibility continuing to be an issue in the cell culture space, it is important to know what variables are being introduced into *in vitro* systems. By testing for both GGT (gamma glutamyl transferase) and IgG (Immunoglobulin G), scientists can know what they are really feeding their cells. This information will help select for product that will lead to more reproducible results. We are also a step closer to being able to determine the age of the animal, which is crucial to identify NBCS or older material from FBS. The ISIA standardization team will be working to set expectations for levels of IgG and Gamma Glutamyl Transferase (GGT) to provide further detailed differentiation of FBS.

We know what you're thinking. Why should you believe us? We don't want you to take our word for it. Recommendations for testing can be found through the industry, including the ISIA ([www.serumindustry.org](http://www.serumindustry.org)), United States Pharmacopeia ([www.usp.org](http://www.usp.org)), EDQM ([www.edqm.eu](http://www.edqm.eu)) and others. FBS suppliers should be publishing more robust certificates of analyses that include, as a standard, the IgG and GGT values of their products. We believe you should be asking yourself some basic questions about FBS before you purchase: Do the results match with other serum suppliers? Are IgG and GGT values within values that I have seen with other FBS? Are the values within industry standard guidelines? Do manufacturers certificates of analysis match independently performed analysis? Is your price quote for FBS significantly lower than what you see on the market? Is that price "too good to be true"? If you have any doubt, test it. A simple inexpensive test can give you peace of mind and at minimum inform you of what you are using. For example, GGT results can be produced in about a day and for less than 30.00 USD.

In the end you have a lot more to lose over #FakeFBS than a supplier has to lose over selling you overpriced NBCS.

Footnotes:

<sup>1</sup> Standardization of Fetal Bovine Serum quality assessment and reporting definitions and sample certificates included retrieved from [www.serumindustry.org/definitions.htm](http://www.serumindustry.org/definitions.htm)

<sup>2</sup> Based on analysis of historic data