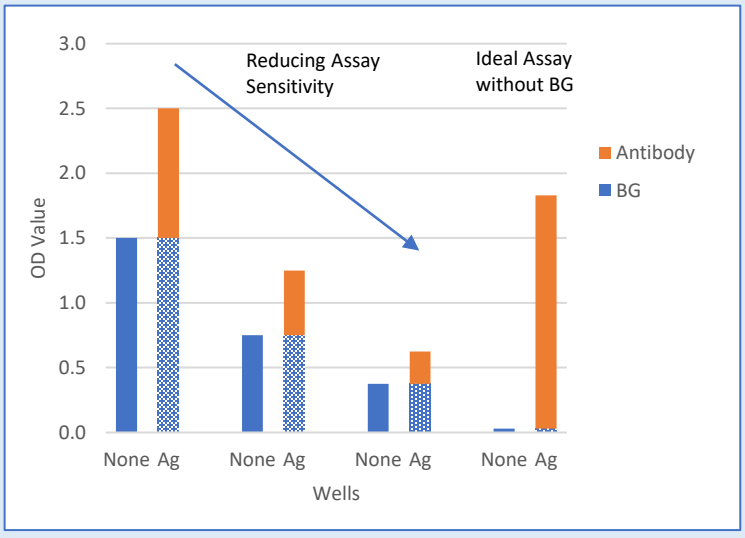
Understanding Background Noise Reactions Caused By Samples to Prevent Further Misuse of the Antibody ELISA System

Key Points

1. Published antibody assay data typically does not subtract false positive reactions caused by samples (background noise reaction).
2. This background (BG) noise reaction caused by the hydrophobic binding of immunoglobulin in samples to plastic surfaces is intense and can exceed the real antibody-antigen reaction.
3. No current blocking agents are capable of preventing this BG noise reaction, although they reduce the blank (BL) values caused by detection antibodies.

ELISAs are widely used to assay antibodies without serious consideration for false positive reactions attributed to the high binding affinity of proteins to plastic surfaces. Among the various non-specific reactions, BL values caused by detection antibodies are considered by all ELISA users, but most BG noise reactions caused by the immunoglobulin samples themselves are often ignored. BG noise reactions are unique to individual samples and vary significantly depending on samples. Without considering this concept, BG noise reaction is included in data and misinterpreted, leading to numerous uncertain conclusions (1). It is imperative to recognize the various types of false reactions involved in ELISAs and to reinvestigate ELISA data to dispel the accumulated misinformed conclusions for future studies on antibodies in biological and medical fields. For more information, please contact Chondrex, Inc. at support@chondrex.com.

Illustration of BG Noise Reaction Effects on Antibody Assay Data in ELISA



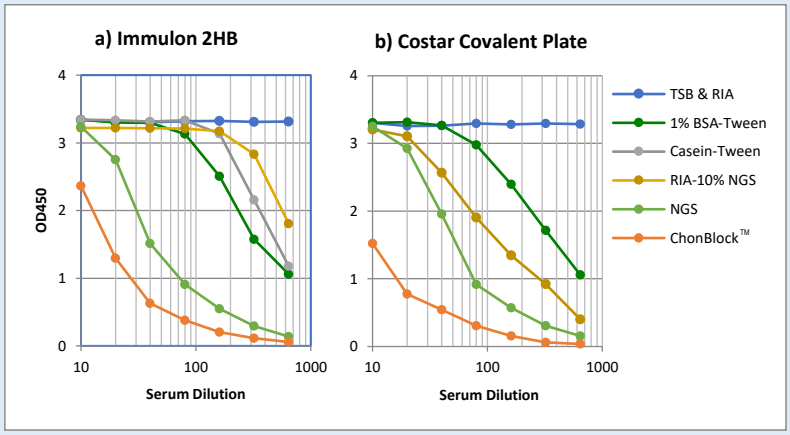
At low serum sample dilution, the BG noise reaction in antigen-coated wells (Ag) is intense and can exceed the antibody-antigen reaction. The BG noise reaction of individual samples can be easily determined in antigen-uncoated (None) wells, but this step is often ignored.

NOTE: Attempts to reduce the BG noise reaction by reducing assay sensitivity do not reduce the BG noise reaction rate.

ChonBlock™ ELISA Buffer

A newly developed buffer system, ChonBlock™, effectively prevents non-specific reactions involved in antibody ELISAs without affecting the antibody-antigen reaction. ChonBlock™ is useful for assaying antibodies against potential pathogenic environmental agents in human and animal sera, for determining the efficacy of various vaccines, and for studying immune function in patients with autoimmune diseases (1). For more information about this product, please contact Chondrex, Inc. at support@chondrex.com.

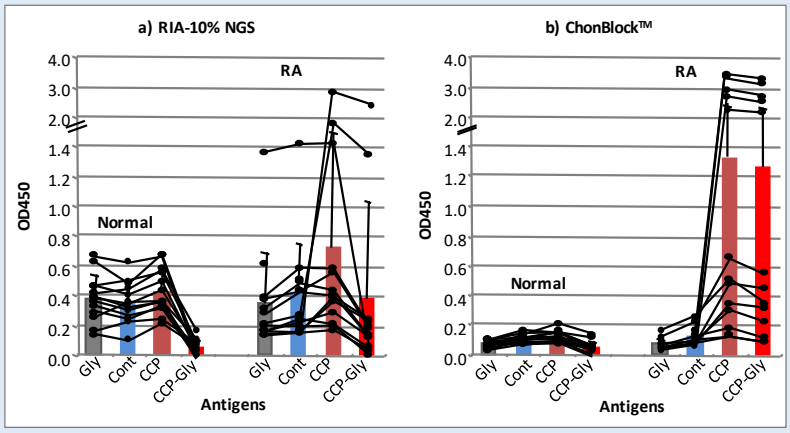
Preventing False Positive BG Noise Reactions with ChonBlock™



Serum from a patient with RA was serially diluted from 1/10 to 1/640 with individual buffers and added to antigen uncoated wells. IgG bound to the plastic surfaces was determined with HRP-conjugated anti-human IgG antibodies.

NOTE: ChonBlock™ effectively blocks BG noise reaction, whereas all current buffer systems fail to prevent it.

Assaying Anti-Cyclic Citrullinated Peptide (CCP) Antibodies



Serum samples from 13 normal controls and 13 patients with RA were diluted at 1/500 with a) RIA-10% normal goat serum (NGS) buffer and b) ChonBlock™ buffer, and added to Glycine (Gly), control peptide (Cont) and CCP-coated wells. The OD values in CCP-coated wells were corrected by subtracting the BG noise OD values obtained in Gly-coated wells to obtain true OD values reflecting anti-CCP antibodies (CCP-Gly).

NOTE: In the RIA-10% NGS buffer system, anti-CCP antibodies were not determined due to the high BG noise reaction.

ChonBlock™	Volume*	Catalog #
Blocking/Sample/Dilution Buffer	100 ml	9068
Detection Antibody Dilution Buffer	100 ml	90681

*ChonBlock™ 1-Liter sizes (Cat # 9068-1L and Cat # 90681-1L) are available upon request. Please inquire at support@chondrex.com.

References

1. Terato *et al.* Preventing intense false positive and negative reactions attributed to the principle of ELISA to reinvestigate antibody studies in autoimmune diseases. *J. Immunol. Methods* **407**, 15-25 (2014).