biosensis® Heterophilic Antibody Blocker

Catalogue Number: BL-005-500

Intended Use: *Sample diluent additive to minimize interference of natural endogenous substances such as heterophilic antibodies (HA) in human samples such as citrate plasma, for use in validated ELISA assays.*

Following ELISA assays in the Biosensis *Rapid*™ ELISA range have been validated to achieve accurate results using BL-005-500.

Product Code	Target	Sample Type
BEK-2212	NGF	Human Citrate Plasma

Other ELISA assays may also benefit from addition of blocker BL-005-500, but require optimization of working concentration and assay validation for accurate results.

For research use only, not for use in clinical or diagnostic procedures

1. Product Description

Two-site sandwich ELISA assays are prone to non-specific components in human-derived samples that interfere with antigen binding, thus causing false-positive or false-negative readings. This can cause inaccurate results, leading to over- or underestimating true target antigen concentrations and incorrect experimental conclusions.

Heterophilic antibodies (HA) are naturally occurring antibodies that can react with immunoglobulins from different species such as mouse, rat, rabbit and sheep amongst others (Boscata and Stuart, 1988). For example Human anti-mouse antibodies (HAMA) specifically bind to mouse antibodies. In comparison, Rheumatoid factor (RF) is an auto-antibody interference meaning that it can react with an individuals own immunoglobulin and also cross-react with ELISA assay antibodies (Kragstrup, *et al*., 2013).

BL-005-500 is a heterophilic antibody blocker that can be used to minimize or eliminate HA cross-reactivity. BL-005 is added to buffers used for dilution of samples known to have interference issues. Validation data demonstrates the benefit of adding BL-005 to sample diluents listed in this datasheet for particular sample types.

2. Materials Provided and Storage Conditions

One vial of BL-005 contains 500 µg of lyophilized, proprietary mixture of immunoglobulins, which can reduce or eliminate false-positive signals from heterophilic antibodies (HA), in sandwich ELISA immunoassays that use mouse assay antibodies.

Reagent	Storage and Stability
Unopened vial	12 months at 2-8°C
Reconstituted blocker	2 weeks at 2-8°C. Unused blocking solution may be aliquoted and stored at -20°C for maximum of 6 months; prevent multiple freeze-thaw cycles

3. Instructions for Use

Briefly spin the vial to collect the lyophilized powder at the bottom of the vial. Reconstitute HA blocker BL-005-500 in 1 mL of Assay Diluent to give an IgG concentration of 500 µg/mL of STOCK blocker solution; mix gently by vortex

Note: The concentration of blocker needed is expected to vary depending upon the target and substrate used. For instance for the target of human NGF and citrate plasma as the substrate, the stock BL-005-500 solution is initially 1/25 (to 20 µg/mL), and then this diluted stock of BL-005-500 is used to further dilute the plasma samples 1/5 to 1/10 before running the samples on the assay. (See BEK-2212-1P/2P insert for complete details).

For other targets and samples types the amount of BL-005-500 required will need to be determined experimentally, typically through a series of spike recovery experiments using control samples and target sources. See Biosensis' "Technical Note #1 - ELISA Assay Validation" [http://www.biosensis.com/documents/enhancedinfo/Technical-Tips-for-ELISA-1.pdf] for a complete explanation of how to begin such validation studies. For use in Biosensis *Rapid*™ ELISA assays, follow the instructions as outlined in each particular kit insert.

4. Sample Data

4.1 NGF Quantification in Human Citrate Plasma

A) Concentration of NGF in Human Citrate Plasma

Four Human citrate plasma samples were diluted 1:5 to 1:40 with the sample diluent containing blocker BL-005-500 and then tested in triplicate in the Biosensis human NGF *Rapid*™ ELISA (BEK-2212). The concentration of NGF detected in the samples is shown in the following table and is the mean of three replicates for four dilutions

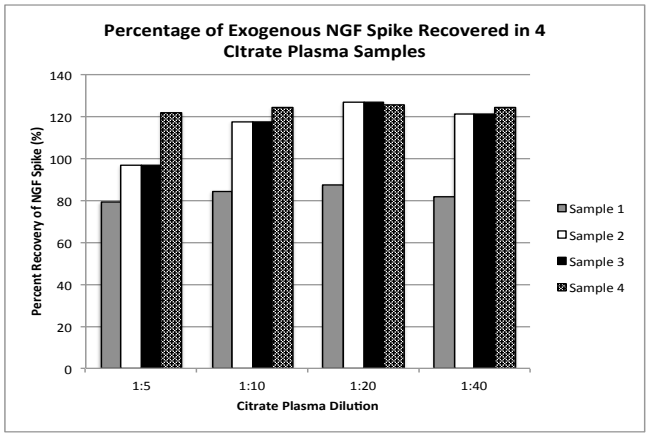
Concentration of NGF in Human Citrate Plasma	
Sample #	(pg/mL)
Plasma 1	147
Plasma 2	88
Plasma 3	69
Plasma 4	408

The table above shows the mean concentration of NGF for dilutions 1/5 to 1/40 for four separate samples, which were diluted in the sample diluent containing the BL-005-500 heterophilic blocker.

B) Spike and Recovery Assay

Four Human serum samples were assayed using the Biosensis human NGF *Rapid*™ ELISA (BEK-2212) with BL-005-500 as sample diluent additive. The plasma samples were diluted at 1:5 to 1:40 and measured in triplicate. The accuracy of results was assessed using a

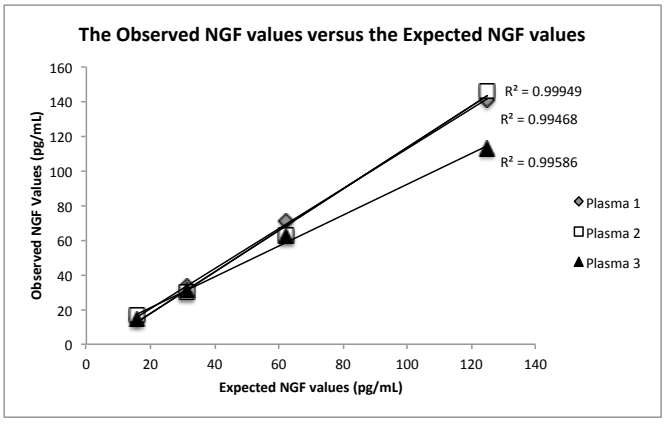
spike-and-recovery assay by spiking exogenous NGF (125 pg/mL) into the plasma samples before dilutions.



The figure above shows the recovery of the spike is acceptable in the four human citrate plasma samples (between 79-127%), with the average recovery of the NGF spike being 104%.

C) Linearity of Dilution Assay

A spike of NGF (125 pg/mL) was added to three separate human citrate plasma samples at a 1:5 dilution in sample diluent containing the Biosensis heterophilic antibody blocker # BL-005-500. These samples were then serially diluted (1:5-1:40) in sample diluent and measured in the Biosensis human NGF *Rapid*™ ELISA (BEK-2212) kit. The observed (measured) NGF values were plotted against the expected NGF values for each plasma sample.



The figure above demonstrates linearity of dilution across the tested dilution range in human citrate-plasma samples, which were diluted in the sample diluent containing the BL-005-500 heterophilic blocker.

Overall, this data shows that NGF in human citrate-plasma can be accurately quantified with a minimum required dilution of 1:5 in presence of HA blocker BL-005-500.

5. Informational References

Boscato and Stuart, **Heterophilic Antibodies: a Problem for All Immunoassays.** Clin Chem. 1988, 34;1:27-33

Kragstrup *et al*., **A simple set of validation steps identifies and removes false results in a sandwich enzyme-linked immunosorbent assay caused by anti-animal IgG antibodies in plasma from arthritis patients.** SpringPlus Open Journal, 2013, 2:263:1-10 http://www.springerplus.com/content/2/1/263