biosensis® Secretory IgA Blocker

Catalogue Number: BL-001-1250

Intended Use: *Sample diluent additive to minimize interference of natural endogenous substances such as secretory immunoglobulin A (sIgA) in human milk samples, for use in validated ELISA assays.*

Following ELISA assays in the Biosensis *Rapid*™ ELISA range have been validated to achieve accurate results using BL-001-1250.

Product Code	Target	Sample Type
BEK-2211	Mature BDNF	Human Milk

Other ELISA assays may also benefit from addition of blocker BL-001-1250, 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). Secretory immunoglobulin A (sIgA) is a type of HA which is predominantly found in high concentrations in mucosal membrane lining, where it exerts critical functions to maintain mucosal membrane immunity (reviewed by Mantis *et al.*, 2011).

Human milk contains natural high levels of sIgA protein, reported to be 1 mg/mL or even higher in some samples (Weaver *et al.*, 1998). These samples like any other immunoglobulin's interfere with sandwich ELISA assays, which can result in elevate sample OD readings, thus causing false-positives and therefore inaccurate results.

BL-001-1250 is a sIgA blocker that can be used to minimize or eliminate sIgA cross-reactivity. BL-001-1250 is added to buffers used for dilution of samples known to have interference issues. Validation data demonstrates the benefit of adding BL-001-1250 to sample diluents listed in this datasheet for particular sample types.

2. Materials Provided and Storage Conditions

One vial of BL-001-1250 contains 1250 µg of lyophilized, proprietary immunoglobulins, which can reduce or eliminate false-positive signals from sIgA antibodies in sandwich ELISA immunoassays.

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

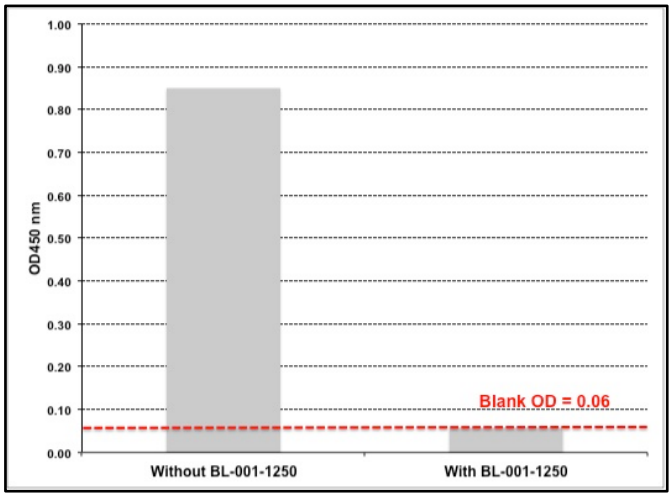
3. Instructions for Use

Briefly spin the vial to collect the lyophilized powder at the bottom of the vial. For use in Biosensis *Rapid*™ ELISA assays, follow the instructions as outlined in each particular kit insert. BL-001-1250 may also be used in other ELISA assays not listed in this datasheet, but achieving accurate results will require determination of optimal working concentration and assay validation. Please visit our website www.biosensis.com to access our Technical Note #1 ("Determining the Accuracy of an ELISA using Spike-and-Recovery and Linearity-of-Dilution Experiments") for assistance.

4. Sample Data

A. Effect of BL-001-1250 on sIgA Cross-Reactivity

The ability of BL-001-1250 to reduce sIgA cross-reactivity was demonstrated in two experiments:
(1) Purified sIgA protein at 250 µg/mL was spiked into assay buffer with and without BL-001-1250. The sIgA concentration was chosen to mimic a 1:4 dilution of a theoretical sample containing high concentrations of 1 mg/mL sIgA.



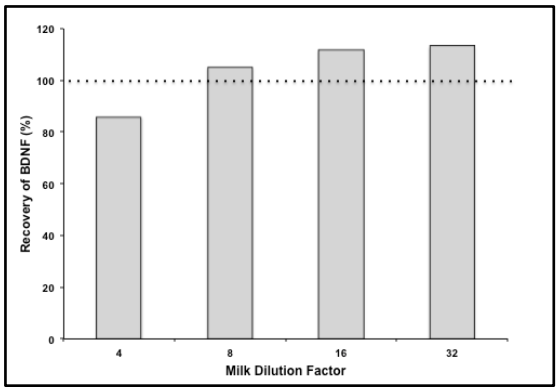
(2) Pooled, normal human milk was assayed for BDNF content, diluting milk in either Assay Diluent A without, or with BL-001-1250.

Sample Dilution	BDNF (pg/mL), dilution corrected	
	Without BL-001-1250	With BL-001-1250
1:4	211	0
1:8	130	0
1:16	0	0
1:32	0	0

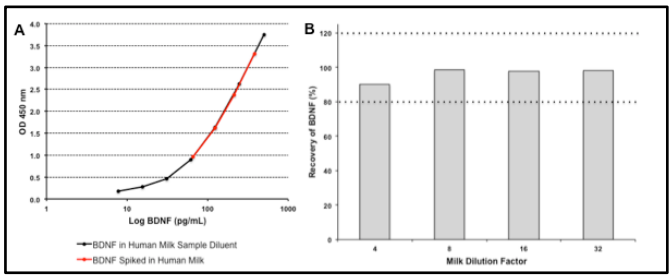
Data shows that BL-001-1250 effectively reduces false-positive OD readings due to sIgA cross-reactivity to background levels.

Linearity and Recovery of BDNF in Human Milk

The effect of sample preparation on BDNF recovery was tested by spiking 1 ng/mL mature BDNF into whole human milk after dilution in Assay Diluent containing BL-001-1250. Prepared human milk was then assayed at 1:4 – 1:32 dilutions (n=2 independent assays) in the BEK-2211 ELISA kit. Recovery values ranged from 86-113% (mean = 104%) demonstrating that BDNF concentrations are not affected by sample preparation.



Sample buffer compatibility with human milk was validated by spiking known amounts of BDNF into 1:4 – 1:32 dilutions of human milk. The ELISA assay showed parallelism between calibration curve and diluted BDNF in human milk (A), as well as excellent recovery values (B) ranging from 90-98% across the dilution range. This demonstrates that a minimum dilution of 1:4 is required in order to assay BDNF in human milk accurately.



Mature BDNF levels in normal pooled human milk were lower than the lowest standard of the calibration curve. Individual milk samples, while not tested, may contain quantifiable levels of BDNF.

5. Informational References

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

Mantis NJ *et al.*, **Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut.** Mucosal Immunol. 2011, 4(6):603-11.

Weaver *et al.*, **Human milk IgA concentrations during the first year of lactation.** Arch Dis Child. 1998, 78:235-239.