VERAVAS
transforming laboratory medicine

VeraPrep Biotin™

REF 500014 and 500015

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NAME OF THE PRODUCT
VeraPrep Biotin™

INTENDED USE
VeraPrep Biotin is a sample pre-treatment system that uses streptavidin-coated magnetic particle technology and VeraMag™ magnetic separation to remove free biotin from an aliquot of plasma or serum. The difference in immunoassay results between non-treated and treated sample is used to detect the presence of biotin interference in samples for immunoassays that are susceptible to biotin interference.

Intended for professional use only.

SUMMARY AND EXPLANATION
Biotin, also known as vitamin B7, is a water-soluble B vitamin often found in multi-vitamins and over the counter health and beauty supplements. *In vitro* laboratory tests that employ streptavidin-biotin binding mechanisms have the potential to be affected by high circulating biotin concentrations. Biotin can be attached through covalent bond to a variety of targets—from large antibodies to steroid hormones—with minimal effect on their specific non-covalent binding with avidin, streptavidin, or NeutrAvidin proteins. Therefore, biotin has been frequently used in the detection systems of immunoassays of different forms.

Immunoassays are generally categorized as either sandwich immunoassays (non-competitive) or competitive inhibition immunoassays. In general, streptavidin-biotin binding is used during assay incubation to couple biotinylated antibodies in sandwich immunoassays, or biotinylated antigens in competitive immunoassays, to streptavidin-coated surfaces. When a biological specimen contains excess biotin, the biotin competes with the biotinylated antibodies or antigens for binding to the streptavidin-coated surfaces, resulting in reduced capture of the biotinylated antibodies or antigens. Excess biotin produces falsely low results in sandwich immunoassays because the assay signal is directly proportional to the analyte concentration. Excess biotin in competitive immunoassays causes falsely elevated results because the assay signal is inversely proportional to the analyte concentration. Specific details of biotin interference have been extensively described in other publications.*(1-13)*

Normal circulating concentrations of biotin derived from the diet and normal metabolism are too low (< 1.2 ng/mL) to interfere with biotinylated immunoassays.*(22)* However, ingestion of high-dose biotin supplements (e.g., 5 mg or higher) can result in significantly elevated blood concentrations that can interfere with commonly used biotinylated immunoassays. In certain medical conditions, extremely high biotin doses (e.g., up to 300 mg per day) can result in serum or plasma biotin levels > 1000 ng/mL, and current trends in biotin consumption may result in biotin levels up to 3,500 ng/mL.(23) According to the FDA, biotin in blood or other samples taken from patients who are ingesting high levels of biotin can cause falsely high or falsely low results in biotin-based immunoassays, depending on the design of the assay.(*14, 21*)

Biotin interference thresholds differ widely among assays, even on a single platform. Tests with biotin interference thresholds < 51 ng/mL are considered high risk tests, or vulnerable immunometric sandwich and competitive methods.(*1*) VeraPrep Biotin is a sample pre-treatment system that can be used to help rule-in or rule-out biotin interference. The system can be used stand-alone for detection of biotin interference or for further evaluation of biotin interference detected by VeraTest Biotin™.

The Standard procedure uses a 1:2 ratio of VeraPrep Biotin reagent to serum or plasma which removes biotin up to 500 ng/mL. The Enhanced procedure uses a 3:2 ratio of VeraPrep Biotin reagent to serum or plasma sample which removes biotin up to 1,500 ng/mL. The Enhanced-Plus procedure uses a 3:1 ratio of VeraPrep Biotin reagent to serum or plasma sample which removes biotin up to 3,500 ng/mL. There is no sample dilution with these procedures.

REAGENTS AND MATERIALS PROVIDED

CONTENT

REAGENT **4mL**

Streptavidin coated superparamagnetic nanoparticles in TRIS buffer and detergent.
Preservative: 0.05% sodium azide.

REF	500014	500015
REAGENT	1x 4mL	5x 4mL

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipetting device(s) capable of delivering 50 µL up to 1000 µL
2. Disposable pipette tips
3. Micro tube 2ml with cap (SARSTEDT Order Number 72.694)
4. Vortex mixer
5. VeraMag (Part No. 500020 or 500021)
6. Timer
7. Laboratory mixer
8. Transfer tube
9. Personal protective equipment

STORAGE AND STABILITY

Upon receipt, store in the box at 2°- 8°C. Refer to the expiration date marked on the vial label.

WARNINGS AND PRECAUTIONS

EXPORT

1. Do not use test components beyond their expiration dates.
2. This product contains sodium azide. For a specific listing, refer to the **REAGENTS AND MATERIALS PROVIDED** section. This material and its container must be disposed of in a safe way.
3. Dispose of all potentially contaminated test components in a biohazard container.
4. If specimens or test components have been stored in a refrigerator, allow them to warm to room temperature before performing the test.
5. Each box contains 1 foam vial holder (donut) to hold the VeraPrep Biotin vial during use and to prevent it from accidently falling over and spilling reagent.
6. Remove the reagent storage solution using VeraMag before adding the sample to prevent sample dilution.
7. VeraPrep Biotin should be used with SARSTEDT tubes (Order Number 72.694). Other tubes types have not been studied.
8. Do not incubate the VeraPrep Biotin reagent on VeraMag without any storage solution or sample.

SPECIMENS COLLECTION AND PREPARATION

Follow manufactures specification for blood collection and serum or plasma preparation.

REAGENT PREPARATION

VeraPrep Biotin has a binding capacity ≥ 1 ng biotin per 1 µL reagent and comprises proprietary superparamagnetic nanoparticles covalently conjugated to Streptavidin. The reagent is in the form of a liquid and must be well mixed prior to use to ensure homogeneous resuspension of the nanoparticles. After > 30 seconds magnetic separation using VeraMag (Part No. 500020 or 500021), the VeraPrep Biotin storage buffer is aspirated and discarded, the serum or plasma sample is added and mixed, and the reagent

incubates with the sample to bind and remove biotin interference from the sample. After a 10 minute incubation (Standard and Enhanced procedures), or a 30 minute incubation (Enhanced-Plus procedure), the reagent is magnetically separated for > 4 minutes using VeraMag and the sample supernatant is aspirated and saved for testing.

STANDARD PROCEDURE

The VeraPrep Biotin Standard procedure uses a 1:2 ratio of VeraPrep Biotin reagent to serum or plasma sample (e.g., 200 µL reagent and 400 µL sample) to deplete biotin interference up to 500 ng/mL. Smaller and larger sample volumes can be used if a 1:2 ratio of reagent:sample is maintained.

Standard Procedure Sample Volumes		
VeraPrep Biotin (µL)	Serum or Plasma (µL)	Samples (Uses per Vial)
50	100	80
100	200	40
200	400	20
300	600	13
400	800	10

Example 1: VeraPrep Biotin Standard procedure to remove biotin interference up to 500 ng/mL from 400 µL serum or plasma:

1. Remove the VeraPrep Biotin reagent vial from storage and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent.
2. Insert the reagent vial in the foam vial holder.
3. Insert an empty Micro tube 2ml (SARSTEDT Order Number 72.694) into the VeraMag magnet until the collar of the tube contacts the magnet frame.
4. Dispense **200 µL** of the well-mixed **reagent** into the empty tube to separate the reagent on the magnet for > 30 seconds to form a reagent pellet.
5. Carefully aspirate and discard all of the storage buffer supernatant (~200 µL) without disturbing the reagent pellet.
6. Dispense **400 µL** of well-mixed serum or plasma **sample** into the tube containing the reagent pellet.
7. Tighten the screw cap on the tube, remove the tube from the magnet, and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent in the sample.
8. Place the tube onto a laboratory mixer at medium speed and **incubate** at room temperature for **10 minutes**.
9. Loosen and remove the screw cap and insert the tube into the magnet until the collar of the tube contacts the magnet frame.
10. Magnetically separate the reagent for > 4 minutes to form a reagent pellet.
11. Carefully aspirate the sample supernatant without disturbing the reagent pellet and dispense the sample into a transfer tube for testing. Note: All of the sample supernatant (~ 400 µL) can be aspirated if this step is performed carefully. If any of the reagent is accidentally aspirated then simply return the sample/reagent mixture to the tube and return to step 10.
12. The sample is now ready for testing.

ENHANCED PROCEDURE

The VeraPrep Biotin Enhanced procedure uses a 3:2 ratio of VeraPrep Biotin reagent to serum or plasma sample (e.g., 600 µL reagent and 400 µL sample) to deplete biotin interference up to 1,500 ng/mL. Smaller and larger sample volumes can be used if a 3:2 ratio of reagent:sample is maintained.

Enhanced Procedure Sample Volumes		
VeraPrep Biotin (µL)	Serum or Plasma (µL)	Samples (Uses per Vial)
150	100	26
300	200	13
600	400	6
900	600	4
1,200	800	3

Example 2: VeraPrep Biotin Enhanced procedure to remove biotin interference up to 1,500 ng/mL from 400 µL serum or plasma:

1. Remove the VeraPrep Biotin reagent vial from storage and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent.
2. Insert the reagent vial in the foam vial holder.
3. Insert an empty Micro tube 2ml (SARSTEDT Order Number 72.694) into the VeraMag magnet until the collar of the tube contacts the magnet frame.
4. Dispense **600 µL** of the well-mixed **reagent** into the empty tube to separate the reagent on the magnet for > 30 seconds to form a reagent pellet.
5. Carefully aspirate and discard all of the storage buffer supernatant (~600 µL) without disturbing the reagent pellet.
6. Dispense **400 µL** of well-mixed serum or plasma **sample** into the tube containing the reagent pellet.
7. Tighten the screw the cap on the tube, remove the tube from the magnet, and vortex for a minimum of seconds at medium speed to mix well and resuspend the reagent in the sample.10 seconds at medium speed to mix well and resuspend the reagent in the sample.
8. Place the tube onto a laboratory mixer at medium speed and **incubate** at room temperature for **10 minutes**.
9. Loosen the screw cap and insert the tube into the magnet until the collar of the tube contacts the magnet frame.
10. Magnetically separate the reagent for > 4 minutes to form a reagent pellet.
11. Carefully aspirate the sample supernatant without disturbing the reagent pellet and dispense the sample into a transfer tube for testing. Note: All of the sample supernatant (~ 400 µL) can be aspirated if this step is performed carefully. If any of the reagent is accidentally aspirated then simply return the sample/reagent mixture to the tube and return to step 10.
12. The sample is now ready for testing.

ENHANCED-PLUS PROCEDURE

The VeraPrep Biotin Enhanced-Plus procedure uses a 3:1 ratio of VeraPrep Biotin reagent to serum or plasma sample (e.g., 1,200 µL reagent and 400 µL sample) to deplete biotin interference up to 3,500 ng/mL. Smaller and larger sample volumes can be used if a 3:1 ratio of reagent: sample is maintained. **Note: the Enhanced-Plus procedure uses a 30 minute sample incubation**.

Enhanced-Plus Procedure Sample Volumes		
VeraPrep Biotin (µL)	Serum or Plasma (µL)	Samples (Uses per Vial)
300	100	13
600	200	6
1,200	400	3
1,800	600	2

Example 3: VeraPrep Biotin Enhanced-Plus procedure to remove biotin interference up to 3,500 ng/mL from 400 µL serum or plasma:

1. Remove the VeraPrep Biotin reagent vial from storage and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent.
2. Insert the reagent vial in the foam vial holder.
3. Insert an empty Micro tube 2ml (SARSTEDT Order Number 72.694) into the VeraMag magnet until the collar of the tube contacts the magnet frame.
4. Dispense **1,200 µL** of the well-mixed **reagent** into the empty tube to separate the reagent on the magnet for > 30 seconds to form a reagent pellet.
5. Carefully aspirate and discard all of the storage buffer supernatant (~1,200 µL) without disturbing the reagent pellet.
6. Dispense **400 µL** of well-mixed serum or plasma **sample** into the tube containing the reagent pellet.
7. Tighten the screw the cap on the tube, remove the tube from the magnet, and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent in the sample.
8. Place the tube onto a laboratory mixer at medium speed and **incubate** at room temperature for **30 minutes**.
9. Loosen the screw cap and insert the tube into the magnet until the collar of the tube contacts the magnet frame.
10. Magnetically separate the reagent for > 4 minutes to form a reagent pellet.
11. Carefully aspirate the sample supernatant without disturbing the reagent pellet and dispense the sample into a transfer tube for testing. Note: All of the sample supernatant (~ 400 µL) can be aspirated if this step is performed carefully. If any of the reagent is accidentally aspirated then simply return the sample/reagent mixture to the tube and return to step 10.
12. The sample is now ready for testing.

LIMITATIONS OF USE

1. VeraPrep Biotin is not intended to replace manufacturer controls provided with the primary assay.
2. The sample may need to be submitted for further evaluation for potential false negative or false positive assay results due to human anti-Streptavidin interference.(15-20)

PERFORMANCE CHARACTERISTICS

Standard Procedure: A study was conducted to demonstrate ability of VeraPrep Biotin to deplete up to 500 ng/mL of interfering biotin. The study consisted of 27 samples: 1 sample per analyte range, 3 analyte ranges (low, medium, high) and 9 analytes (Roche Elecsys® TSH, Elecsys® FT4 II, Elecsys® Troponin T Gen 5 STAT, Elecsys® proBNP II, Elecsys® C-peptide, Elecsys® FSH, Elecsys® LH, Elecsys® Progesterone III, and Elecsys® T3). Each sample was spiked with a target of 500 ng/mL biotin. Biotin concentrations were quantitatively determined by LC-MS/MS and ranged from 282 ng/mL to 501 ng/mL. The Roche assay results were compared between untreated biotin spiked samples (Untreated) and VeraPrep Biotin treated samples (Treated). In the presence of biotin interference greater than the assay-specific biotin interference threshold, analyte concentrations falsely decrease in sandwich assays and falsely increase in competitive assays. When the biotin-spiked samples were treated using VeraPrep Biotin, the analyte % change was significantly increased for the 18 sandwich assay samples (**Figure 1A**) and significantly decreased for the 6 competitive assay samples (**Figure 1B**). This confirms the presence of biotin interference in the untreated assay results.

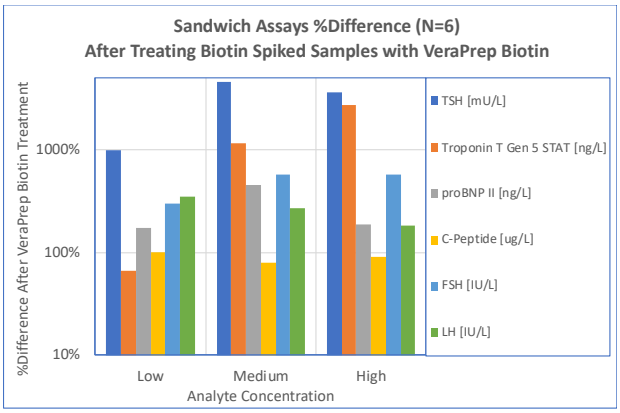

Figure 1A

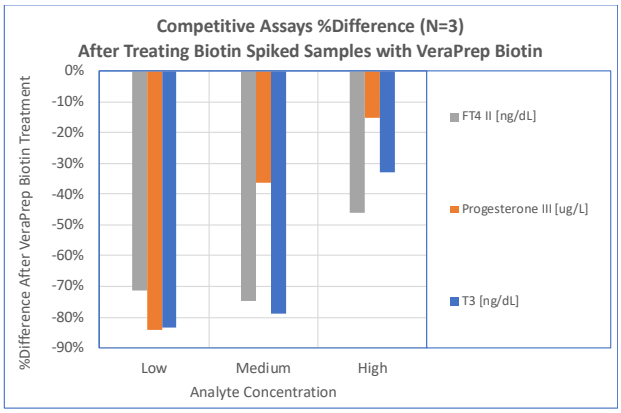

Figure 1B

Standard Procedure: A low biotin study was conducted to demonstrate the VeraPrep Biotin Standard Procedure does not introduce a matrix effect in the absence of interfering biotin. The study consisted of 81 samples: 3 samples per analyte range, 3 analyte ranges (low, medium, high), and 9 analytes (Roche Elecsys® TSH, Elecsys® FT4 II, Elecsys® Troponin T Gen 5 STAT, Elecsys® proBNP II, Elecsys® C-peptide, Elecsys® FSH, Elecsys® LH, Elecsys® Progesterone III, and Elecsys® T3). Endogenous biotin concentrations were quantitatively determined by LC-MS/MS and ranged from 0.1 ng/mL to only 1.0 ng/mL. The Roche assay results were compared between untreated samples (Neat) and VeraPrep Biotin treated samples (Treated). Mean results were reported for each analyte level for each analyte. The low % difference shows no matrix effect or assay bias from VeraPrep Biotin sample treatment (**Figures 2A and 2B**). Of the 27 results, 26 had absolute % differences <10% between Neat and Treated. One sample had 17% difference (Neat = 0.6 ng/dL; Treated = 0.7 ng/dL). This higher % difference was due to the very low analyte concentration and the mathematical impact of such a small difference in values.

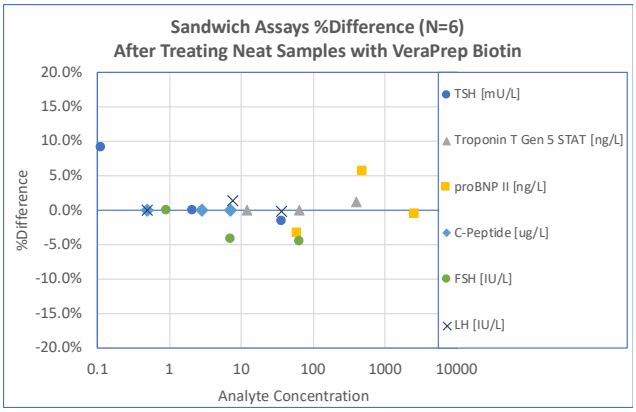

Figure 2A

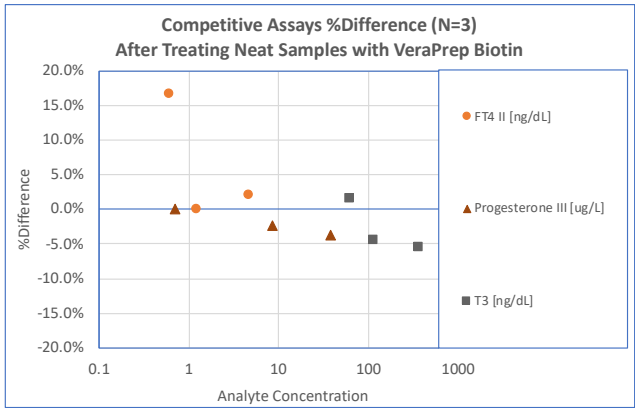

Figure 2B

Enhanced-Plus Procedure: A study was conducted using the Enhanced-Plus procedure to demonstrate ability of VeraPrep Biotin to deplete up to 3,500 ng/mL of interfering biotin.[21] The study consisted of 30 samples: 10 samples and 3 analytes (Roche Elecsys® BRAHMS PCT, Elecsys® proBNP II and Elecsys® Troponin T hs). Each sample was spiked with 3,500 ng/mL biotin, treated by VeraPrep Biotin and retested by the 3 assays. When the 3,500 ng/mL biotin-spiked samples were depleted using VeraPrep Biotin, the % analyte changes for the 30 samples showed significant increase beyond the individual assays' claimed precision (**Figure 3**). This demonstrates the ability of the VeraPrep Biotin to detect very high levels of biotin interference.

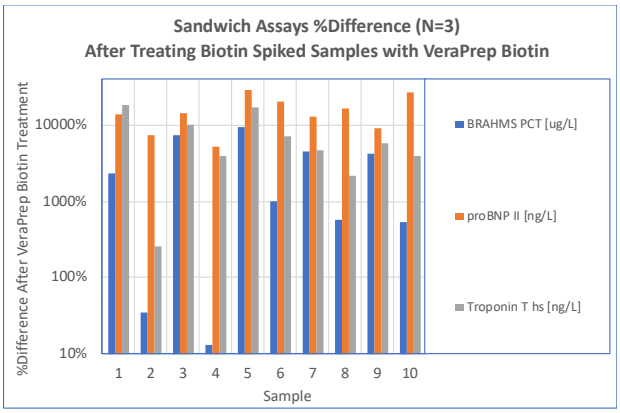

Figure 3

Enhanced-Plus Procedure: A low biotin study was conducted to demonstrate the VeraPrep Biotin Enhanced-Plus Procedure (6-fold more reagent and a 20 minute longer incubation than the Standard Procedure) does not introduce a matrix effect in the absence of interfering biotin. Ten (10) neat samples were tested with 3 sandwich assays (Roche Elecsys® BRAHMS PCT, Elecsys® proBNP II and Elecsys® Troponin T hs). The samples were treated with the VeraPrep Biotin Enhanced-Plus procedure and retested with the 3 assays after treatment. The low % difference shows no matrix effect or assay bias (**Figure 4**).

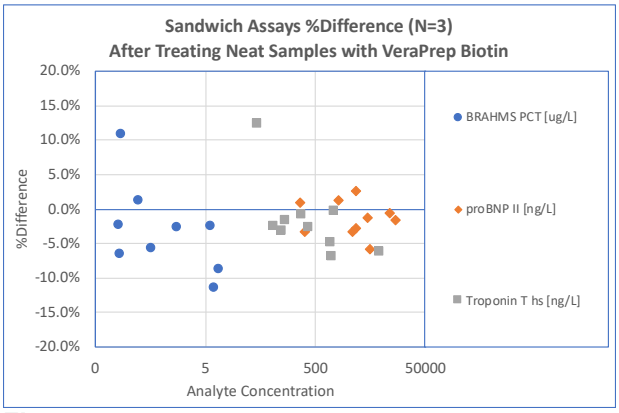

Figure 4

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EC REP

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CONTENT

REAGENT **4mL**

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