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VeraPrep Biotin™

Part No. 500014 and 500015

For Research Use Only

NAME OF THE PRODUCT

VeraPrep Biotin

INTENDED USE

VeraPrep Biotin is a research use only sample pre-treatment reagent that removes biotin interference from a serum or plasma sample. It is used in conjunction with a non-treated sample to investigate possible interference from biotin in immunoassays.

VeraPrep Biotin is not intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae.

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 (< 1.1 ng/mL) to interfere with biotinylated immunoassays. 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. 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 research use only sample pre-treatment reagent that can be used to help rule-in or rule-out biotin interference. The standard procedure uses a 1:2 ratio of VeraPrep Biotin reagent to serum or plasma, the enhanced procedure uses a 3:2 ratio of VeraPrep Biotin reagent to serum or plasma sample, and the enhanced-plus procedure uses a 3:1 ratio of VeraPrep Biotin reagent to serum or plasma sample, to remove biotin up to 500 ng/mL,1,500 ng/mL or 3,500 ng/mL, respectively, without sample dilution.

REAGENT

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.

REAGENTS AND MATERIALS PROVIDED

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

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. The shelf life is approximately six (6) months. Refer to the expiration dates marked on the vial label.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Do not use test components beyond their expiration dates.
3. 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.
4. Dispose of all potentially contaminated test components in a biohazard container.
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 VeraBind 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.

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 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

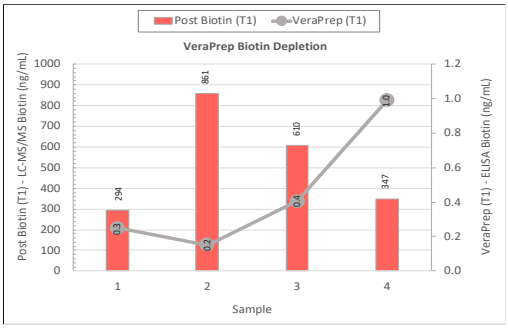
1. For Research Use Only. Not for use in diagnostic procedures.
2. VeraPrep Biotin is not intended to replace manufacturer controls provided with the primary assay.
3. The sample may need to be submitted for further research for potential false negative or false positive assay results due to human anti-Streptavidin interference.(*15-20*)

PERFORMANCE CHARACTERISTICS

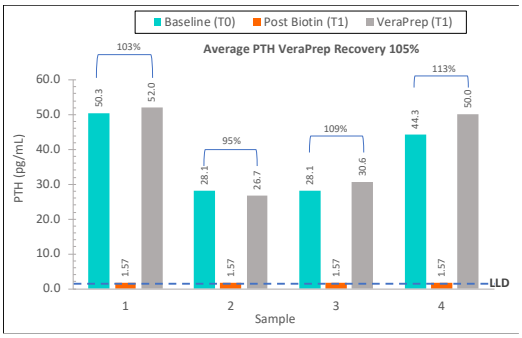
An internal study was completed to demonstrate the ability of VeraPrep Biotin to remove high levels of biotin from serum samples and to rule-in biotin interference in a PTH ELISA test susceptible to biotin interference (i.e. Streptavidin coated microtiter plate wells, sample added to microtiter plate wells prior to the biotinylated capture antibody).

1. Four apparently healthy adult volunteers had fasting baseline serum samples collected, ingested 100-200 mg of over the counter (OTC) biotin, and had serum samples collected again 1 hour post-biotin ingestion. All serum samples were aliquoted and stored frozen at -80° Celsius until testing.
2. The baseline serum samples were tested by the PTH Intact ELISA (DRG PTH Intact ELISA, Part No. EIA-3645). PTH values ranged from 28.1 to 50.3 pg/mL.
3. The 1 hour post-biotin ingestion samples were tested by the PTH Intact ELISA. All PTH results were < 1.57 pg/mL, or below the lower limit of detection (LLD).
4. Endogenous biotin levels in each sample were determined by LC-MS/MS (Department of Lab Medicine, University of Washington Medical Center, Seattle, WA). Biotin concentrations ranged from 294 to 861 ng/mL.
5. Samples 1 and 4 had biotin levels < 500 ng/mL and were pre-treated using the VeraPrep Biotin standard procedure.
6. Samples 2 and 3 had biotin levels greater than 500 ng/mL and were pre-treated using the VeraPrep Biotin enhanced procedure.
7. To verify biotin interference removal, VeraPrep Biotin pre-treated samples were tested using the Immundiagnostik IDK® Biotin ELISA kit (Part No. K8141, measuring range of 48.1 – 1,100 ng/L).

Biotin concentrations ranged from 0.2 to 1.0 ng/mL, or within normal plasma levels (0.2 – 1.1 ng/mL).



8. Immediately after VeraPrep Biotin pre-treatment of Samples 1-4, PTH values were measured using the PTH Intact ELISA. PTH values ranged from 26.7 to 52.0 pg/mL.
9. The 1 hour post-biotin ingestion samples had high levels of biotin interference per LC-MS/MS (294 to 861 ng/mL) and undetectable PTH values by the PTH Intact ELISA (< 1.57 pg/mL).
10. The 1 hour post-biotin ingestion samples pre-treated with VeraPrep Biotin had physiologically normal biotin values per the Biotin ELISA kit (< 1.1 ng/mL) and normal PTH values by the PTH Intact ELISA (26.7 to 52.0 pg/mL).



11. When comparing VeraPrep Biotin treated samples (grey bars) to the baseline PTH values (blue bars) the results recovered from 95 to 113% (mean recovery of 105%).

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