

VeraPrep ABAS™

REF 500050 and 500051



NAME OF THE PRODUCT

VeraPrep ABAS™

INTENDED USE

VeraPrep ABAS is a sample pre-treatment system that uses biotinylated inactive streptavidin-coated magnetic particle technology and VeraMag™ magnetic separation to remove anti-biotin and anti-streptavidin interference 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 heterophilic interference in samples for immunoassays that are susceptible to anti-streptavidin and anti-biotin interference.

Intended for professional use only.

SUMMARY AND EXPLANATION

Biotin, also known as Vitamin B7, Vitamin H and Coenzyme R, is a water-soluble vitamin often found in high doses in over the counter (OTC) dietary supplements, multi-vitamins, and prenatal vitamins. Biotin is marketed for health & beauty including hair, skin and nail growth, as well as weight loss. It is also given to patients at high therapeutic doses to treat certain medical conditions. 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.(1-11)

Immunoassays are generally categorized as either sandwich immunoassays (non-competitive) or competitive inhibition immunoassays. In general, streptavidin-biotin binding is used during the assay incubation to couple biotinylated antibodies in sandwich immunoassays, or biotinylated antigens in competitive immunoassays, to streptavidin-coated surfaces.(4-7)

Anti-biotin and anti-streptavidin (**ABAS**) antibodies and proteins may interfere with *In vitro* laboratory tests that employ streptavidin-biotin binding mechanisms. Similar to biotin interference which causes a decreased test signal and false low or false high test result depending on the assay design and format, anti-biotin and anti-streptavidin interference also results in a decreased test signal but via different mechanisms. While anti-biotin and anti-streptavidin interferences have been reported in the literature, it has been difficult to detect and confirm these specific interference mechanisms or to differentiate them from biotin interference.(12-25)

When a sample contains anti-biotin antibodies and proteins, the anti-biotin interference can bind to biotinylated antibodies/antigens (conjugated biotin) used in the test design or assay format and sterically block or impair accessibility of the conjugated biotin to bind to the streptavidin solid phase or other anti-biotin capture moiety. If conjugated biotin can no longer freely bind the anti-biotin capture moiety, just like biotin interference, anti-biotin interference will result in a false low assay signal and may result in a false low dose (sandwich assay) or false high dose (competitive inhibition assay).(12-13)

When a sample contains anti-streptavidin antibodies and proteins, the anti-streptavidin interference can bind to streptavidin or its polypeptide chains and sterically block or impair conjugated biotin from binding to streptavidin's biotin binding sites. If streptavidin can no longer freely bind the biotinylated antibody, protein, or antigen used in the test design or assay format, just like biotin interference, streptavidin interference will result in a false low assay signal and can result in a false low dose (sandwich assay) or false high dose (competitive inhibition assay).(14-25)


VeraPrep ABAS is a research use only sample pre-treatment reagent that can be used to help rule-in or rule-out anti-biotin and anti-streptavidin interference. It uses a 15 minute procedure to remove anti-biotin and anti-streptavidin antibodies and proteins in serum or plasma without sample dilution.

REAGENTS AND MATERIALS PROVIDED

CONTENT

REAGENT

4 mL
Biotinylated inactive streptavidin-coated superparamagnetic nanoparticles in TRIS buffer and detergent. Preservative: 0.05% sodium azide.

REF	500050	500051
REAGENT	1x 4mL	5x 4mL
	20	100

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 ABAS reagent vial during use and to prevent it from accidentally falling over and spilling reagent.
6. Remove the reagent storage solution using VeraMag before adding the sample to prevent sample dilution.
7. VeraPrep Interference should be used with SARSTEDT tubes (Order Number 72.694). Other tubes types have not been studied.
8. Do not incubate the VeraPrep ABAS reagent on VeraMag without any storage solution or sample.

EXPORT

SPECIMENS COLLECTION AND PREPARATION

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

REAGENT PREPARATION

VeraPrep ABAS reagent contains proprietary superparamagnetic nanoparticles covalently conjugated to biotinylated inactive Streptavidin. After > 30 seconds magnetic separation using VeraMag™ (Part No. 40020), the VeraPrep ABAS 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 anti-biotin and anti-streptavidin interference from the sample. After a 10 minute incubation, the reagent is magnetically separated for > 4 minutes using VeraMag and the sample supernatant is aspirated and saved for testing. Each VeraPrep ABAS vial contains enough reagent to pre-treat 20 different 400 µL samples using the standard procedure. The reagent is in the form of a liquid and must be well mixed prior to use to ensure homogeneous resuspension of the nanoparticles.

STANDARD PROCEDURE

The standard VeraPrep ABAS procedure uses a 1:2 ratio of VeraPrep ABAS reagent to serum or plasma sample, or 200 µL reagent and 400 µL sample, to remove anti-biotin and anti-streptavidin interference. Smaller and larger sample volumes can be used if a 1:2 ratio of reagent:sample is maintained.

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

Example 1: VeraPrep ABAS Standard Procedure:

1. Remove the VeraPrep ABAS 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

Anti-biotin and anti-streptavidin antibodies and proteins can have sample-specific binding affinities. A longer incubation time with VeraPrep ABAS reagent may be required to significantly reduce an anti-biotin or anti-streptavidin interference with a weak binding affinity.

The enhanced procedure increases the sample incubation time from 10 minutes (see Step 8 in the Standard Procedure) to **30 minutes** with the VeraPrep ABAS reagent.

LIMITATIONS OF USE

VeraPrep ABAS is not intended to replace manufacturer controls provided with the primary assay.

INTERFERENCE TROUBLESHOOTING

VeraPrep ABAS can be used with VeraPrep Biotin™ (Part No. 500014 and 500015) to rule-in/rule-out biotin related interference and determine which interference mechanism is likely present in a sample.

1. Two different aliquots of the sample are pretreated with VeraPrep Biotin (Part No. 500014 or 500015) and VeraPrep ABAS and re-tested.
2. The VeraPrep Biotin and VeraPrep ABAS test results are compared to the un-treated sample test result.
3. If a VeraPrep Biotin or VeraPrep ABAS test result is similar to the un-treated sample test result interference is unlikely or “-”.
4. If a VeraPrep Biotin or VeraPrep ABAS test result is significantly different than the un-treated sample test result interference is likely or “+”.
5. If both results are “-” interference can be ruled-out. If either result is “+” the corresponding interference mechanism can be ruled-in. If both results are “+”, the sample may have biotin interference, anti-streptavidin interference, or both mechanisms simultaneously (see “Detect and Determine Interference Mechanism” Table).(14,18)

Detect and Determine Interference Mechanism				
	Result 1	Result 2	Result 3	Result 4
VeraPrep Biotin	-	+	-	+
VeraPrep ABAS	-	-	+	+
Biotin Interference	No	Yes	No	Possible
Anti-Biotin Interference	No	No	Yes	No
Anti-Streptavidin Interference	No	No	No	Possible
Interference Likely	Rule-Out	Rule-In	Rule-In	Rule-In

PERFORMANCE CHARACTERISTICS

A study was conducted to demonstrate ability of VeraPrep ABAS to remove anti-biotin IgG interference, anti-streptavidin IgG interference, and a mixture of anti-biotin IgG interference and anti-streptavidin IgG interference, but to not bind any free biotin interference, using the Standard Procedure (1:2 ratio of reagent:sample). The study consisted of 15 samples: 3 serum samples per set, 5 sets, and 1 analyte (DRG PTH Intact ELISA, Part No. EIA-3645). The biotin sample was spiked with a target of 250 ng/mL biotin, the anti-biotin IgG sample was spiked with a target of 16.5 µg/mL, the anti-streptavidin samples was spiked with a target of 16.5 µg/mL, and the anti-biotin/anti-streptavidin sample was spiked with a target of 8.25 µg/mL of each IgG (1:1 mixture Anti-Biotin IgG and Anti-SAV IgG).

- **Set 1 (None)**
 - Baseline: PTH sample 1
 - Interferent Spike: Baseline spiked with PBS (vehicle control)
 - VeraPrep ABAS: Baseline treated with reagent

- **Set 2 (Biotin)**
 - Baseline: PTH sample 2
 - Interferent Spike: Baseline spiked with biotin
 - VeraPrep ABAS: Baseline spiked with biotin and treated with reagent
- **Set 3 (Anti-Biotin IgG)**
 - Baseline: PTH sample 3
 - Interferent Spike: Baseline spiked with anti-biotin IgG
 - VeraPrep ABAS: Baseline spiked with anti-biotin IgG and treated with reagent
- **Set 4 (Anti-SAV IgG)**
 - Baseline: PTH sample 4
 - Interferent Spike: Baseline spiked with anti-streptavidin IgG
 - VeraPrep ABAS: Baseline spiked with anti-streptavidin IgG and treated with reagent
- **Set 5 (Anti-Biotin/SAV IgG)**
 - Baseline: PTH sample 5
 - Interferent Spike: Baseline spiked with anti-biotin IgG and anti-streptavidin IgG
 - VeraPrep ABAS: Baseline spiked with anti-biotin IgG and anti-streptavidin IgG and treated with reagent

The DRG PTH Intact ELISA assay results were compared between untreated samples (Untreated) and VeraPrep ABAS treated samples (Treated)(see Table 1).

- VeraPrep ABAS treatment of the Baseline sample without any interference (None) was only +1.3% different than the untreated sample. This demonstrates reagent neutrality and that VeraPrep ABAS treatment did not introduce any sample dilution or matrix effect in the PTH assay.
- The Biotin spike caused significant interference in the PTH assay and resulted in a -88% decrease in PTH value. When the Biotin spike was treated with VeraPrep ABAS the PTH result did not significantly change and was only -2.3% different. This is expected as VeraPrep ABAS does not bind or remove free biotin.
- The Anti-Biotin IgG spike caused significant interference in the PTH assay and resulted in a -97% decrease in PTH value. When the Anti-Biotin IgG spike was treated with VeraPrep ABAS the PTH result significantly changed and was +3,546% different. This is expected as VeraPrep ABAS was designed to bind and deplete anti-biotin antibody interference. The treated PTH result was only -1.5% difference from Baseline and demonstrates VeraPrep ABAS removed the majority of the 16.5 µg/mL anti-biotin IgG spiked in this sample.
- The Anti-streptavidin IgG spike caused significant interference in the PTH assay and resulted in a -74% decrease in PTH value. When the Anti-streptavidin IgG spike was treated with VeraPrep ABAS the PTH result significantly changed and was +253% different. This is expected as VeraPrep ABAS was designed to bind and deplete anti-streptavidin antibody interference. The treated PTH result was only -8.2% difference from Baseline and demonstrates VeraPrep ABAS removed the majority of the 16.5 µg/mL anti-streptavidin IgG spiked in this sample.
- The 1:1 mixture Anti-Biotin IgG and Anti-streptavidin IgG spike caused significant interference in the PTH assay and resulted in a -81% decrease in PTH value. When the Anti-Biotin IgG and Anti-streptavidin IgG spike was treated with VeraPrep ABAS the result significantly changed and was +379% different. This is expected as VeraPrep ABAS was designed to bind and deplete both anti-biotin antibody interference and anti-streptavidin antibody interference. The treated PTH result was only -9.9% difference from Baseline and demonstrates VeraPrep ABAS removed the majority of the 8.25 µg/mL Anti-biotin IgG and 8.25 µg/mL Anti-streptavidin IgG spiked in this sample. These data also demonstrate the ability of VeraPrep ABAS to simultaneously bind and deplete both interference mechanisms from the same sample.

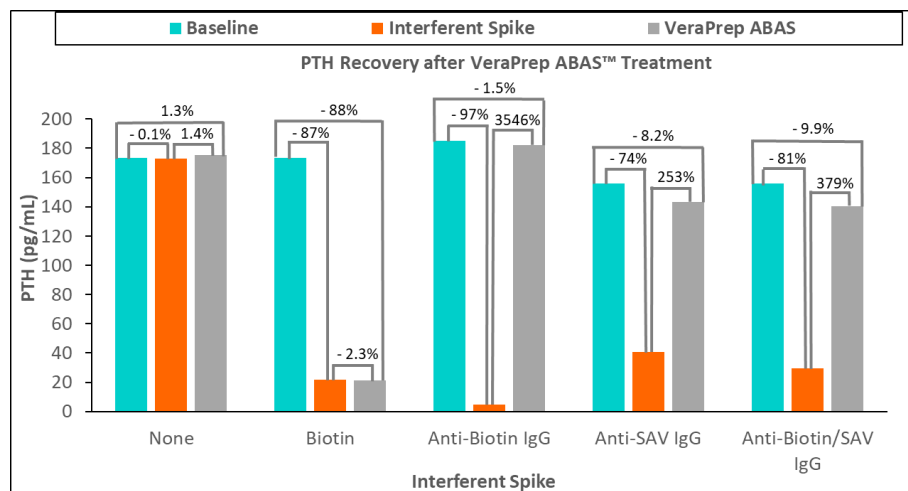


Table 1

REFERENCES

1. Frame IJ, Joshi PH, Mwangi C, Gondolas I, De Lemos JA, Das SR, Sarode R, Balani J, Apple FS, Muthukumar A. Susceptibility of Cardiac Troponin Assays to Biotin Interference. *Am J Clin Pathol*. 2019 Apr 2;151(5):486-493.
2. Wild, D. Biotin interference: answering questions, reducing the risk. *CAP Today*. November 2018. <http://captodayonline.com/biotin-interference-answering-questions-reducing-the-risk/>
3. Katzman BM, Lueke AJ, Donato LJ, Jaffe AS, Baumann NA. Prevalence of biotin supplement usage in outpatients and plasma biotin concentrations in patients presenting to the emergency department. *Clin Biochem*. 2018 Sep;60:11-16
4. Colon, P.J., Green, D.N. Biotin Interference in Clinical Immunoassays. *JALM*. 2018; 02(06): 941-951. DOI: 10.1373/jalm.2017.024257
5. Kirkwood, Julie. Meeting the Biotin Challenge. *Clinical Laboratory News*. January, 2018. <https://www.aacc.org/publications/cln/articles/2018/janfeb/meeting-the-biotin-challenge>
6. Chun, Kelly Y. Biotin Interference in Diagnostic Tests. *Clin Chem*. 2017 Feb;63(2):619-620
7. Samarasinghe S, Meah F, Singh V, Basit A, Emanuele N, Emanuele MA, Mazhari A, Holmes EW. Biotin Interference with Routine Clinical Immunoassays: Understand the Causes and Mitigate the Risks. *Endocr Pract*. 2017 Aug;23(8):989-998
8. Lam L, Kyle CV. A simple method to detect biotin interference on immunoassays. *Clin Chem Lab Med*. 2017 May 1;55(6):e104-e106.
9. Trambas C, Lu Z, Yen T, Sikaris K. Depletion of biotin using streptavidin-coated microparticles: a validated solution to the problem of biotin interference in streptavidin-biotin immunoassays. *Ann Clin Biochem*. 2018 Mar;55(2):216-226.
10. Piketty ML, Prie D, Sedel F, Bernard D, Hercend C, Chanson P, Souberbielle JC. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. *Clin Chem Lab Med*. 2017 May 1;55(6):817-825.
11. Barbesino, G. The Unintended Consequences of Biotin Supplementation: Spurious Immunoassay Results Lead to Misdiagnoses. *Clinical Laboratory News, Bench Matters*. December 2016. <https://www.aacc.org/publications/cln/articles/2016/december/bench-matters-december-2016>
12. Tytgat HL, Schoofs G, Driesen M, Proost P, Van Damme EJ, Vanderleyden J, Lebeer S. Endogenous biotin-binding proteins: an overlooked factor causing false positives in streptavidin-based protein detection. *Microb Biotechnol*. 2015 Jan;8(1):164-8
13. Chen T, Hedman L, Mattila PS, Jartti L, Jartti T, Ruuskanen O, Söderlund-Venermo M, Hedman K. Biotin IgM antibodies in human blood: a previously unknown factor eliciting false results in biotinylation-based immunoassays. *PLoS One*. 2012;7(8):e42376.
14. Harsch IA, Konturek PC, Böer K, Reinhöfer M. Implausible elevation of peripheral thyroid hormones during therapy with a protein supplement. *Clin Chem Lab Med*. 2017 Aug 28;55(9):e197-e198.
15. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. *Arch Pathol Lab Med*; 2013. 137(8):1141-6.
16. Chater KF. Recent advances in understanding Streptomyces. *F1000Res*. 2016 Nov 30;5:2795. doi: 10.12688/f1000research.9534.1.
17. Peltier L, Massart C, Moineau MP, Delhostal A, Roudaut N. Anti-streptavidin interferences in Roche thyroid immunoassays: a case report. *Clin Chem Lab Med*; 2016. 54(1):e11-4.

18. Lam L, Bagg W, Smith G, Chiu WW, Middleditch MJ, Lim JC, Kyle CV. Apparent Hyperthyroidism Caused by Biotin-Like Interference from IgM Anti-Streptavidin Antibodies. *Thyroid*; 2018. 28(8):1063-1067.
19. Favresse J, Lardinois B, Nassogne MC, Preumont V, Maiter D, Gruson D. Anti-streptavidin antibodies mimicking heterophilic antibodies in thyroid function tests. *Clin Chem Lab Med*; 2018. 56(7): e160-e163.
20. Berth M, Willaert S, De Ridder C. Anti-streptavidin IgG antibody interference in anti-cyclic citrullinated peptide (CCP) IgG antibody assays is a rare but important cause of false-positive anti-CCP results. *Clin Chem Lab Med*; 2018. 56(8): 1263-1268.
21. Favresse J, Burlacu MC, Maiter D, Gruson D. Interferences With Thyroid Function Immunoassays: Clinical Implications and Detection Algorithm. *Endocr Rev*. 2018 Oct 1;39(5):830-850.
22. Bayart JL, Favresse J, Melnik E, Lardinois B, Fillee C, Maiter D, et al. Erroneous thyroid and steroid hormones profile due to anti-streptavidin antibodies. *Clin Chem Lab Med*; 2019. 57(10): e255–e258.
23. Verougstaete N, Berth M, Vanechoutte M, Delanghe J, Callewaert N. Interference of anti-streptavidin antibodies in immunoassays: a very rare phenomenon or a more common finding? *Clin Chem Lab Med*; 2019. 1064, eISSN 1437-4331, ISSN 1434-6621.
24. Wouters Y, Oosterbos J, Reynaert N, Penders J. Alarmed by misleading interference in free T3 and free T4 assays: a new case of anti-streptavidin antibodies. *Clin Chem Lab Med*. 58(3): e69-e71.
25. Robier C, Kolbus N, Demel U. Anti-streptavidin antibodies as a cause of false-positive results of streptavidin-based autoantibody assays. *Clin Chem Lab Med*. 58(1):e5-e7.

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REAGENT

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