VeraPrep™ Interference

REF 500070 and 500071

RUO

NAME OF THE PRODUCT
VeraPrep™ Interference powered by TRU BLOCK®

Intended Use
VeraPrep Interference is a sample screening system that uses magnetic particle technology and magnetic separation to detect biotin and immunoglobulin interference in plasma or serum. Differences in immunoassay results between VeraPrep Interference treated and non-treated sample aliquots assist in the detection of biotin interference and heterophilic antibody interference in samples for immunoassays that are susceptible to biotin and immunoglobulin interference.

No analyte-specific quantitative results are reported.

For Research Use Only.

Not for use in diagnostic procedures.

Summary and Explanation
Immunoassays are based on antigen-antibody binding to generate an assay measurement. Certain types of compounds may alter the immunoassay's critical binding actions and may interfere with assay measurement.

Biotin, anti-biotin immunoglobulins, and anti-streptavidin immunoglobulins may interfere with *In vitro* laboratory tests that employ streptavidin-biotin binding mechanisms. 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. Anti-streptavidin and anti-biotin immunoglobulins 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-streptavidin and anti-biotin immunoglobulin interference also results in a decreased test signal but via different mechanisms. HAAA's (human anti-animal antibodies) may be produced in response to therapeutic agents that include animal-derived monoclonal antibodies. HAMA (Human anti-mouse antibodies) constitute a subset of HAAA and is the most common of this interfering antibody type. ***(1-9)***

Reagents and Materials Provided

CONTENT

REAGENT **5mL** Streptavidin coated superparamagnetic nanoparticles coupled with TRU BLOCK® and animal antibodies (mouse, goat, sheep, rabbit and bovine) in TRIS buffer and detergent. Preservative: 0.05% sodium azide.

REF	500070	500071
REAGENT	1x 5mL	5x 5mL
Σ	50	250

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. Strong magnet, such as 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

1. For Research Use Only. Not for use in diagnostic procedures.
2. Do not use reagent beyond its expiration date.
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. If specimens or reagent has been stored in a refrigerator, allow it to warm to room temperature before performing the Standard Procedure or Enhanced Procedure.
6. Each box contains 1 foam vial holder (donut) to hold the VeraPrep Interference reagent vial during use and to prevent it from accidently falling over and spilling reagent.
7. Remove the reagent storage solution using a strong magnet, such as VeraMag™ (Part No. 500020 or 500021), before adding the sample to prevent sample dilution.
8. VeraPrep Interference should be used with SARSTEDT tubes (Order Number 72.694). Other tubes types have not been studied.
9. Do not incubate the VeraPrep Interference reagent on a strong magnet without any storage solution or sample.

Reagent Preparation

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 VeraPrep Interference Standard procedure uses a 1:2 ratio of VeraPrep Interference reagent to serum or plasma sample (e.g., 100 µL reagent and 200 µL sample) to deplete biotin interference up to 100 ng/mL, anti-streptavidin and HAMA interference up to 10 µg/mL, and both anti-biotin antibody and HAAA interference up to 5 µg/mL. Smaller and larger sample volumes can be used if a 1:2 ratio of reagent: sample is maintained.

Standard Procedure Sample Volumes		
VeraPrep Interference (µL)	Serum or Plasma (µL)	Samples (Uses per Vial)
50	100	100
100	**200**	**50**
250	500	20

Example 1: VeraPrep Interference Standard Procedure:

1. Remove the VeraPrep Interference reagent vial from storage and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent.
2. 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.
3. Dispense **100 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.
4. Carefully aspirate and discard all of the storage buffer supernatant (~100 µL) without disturbing the reagent pellet.
5. Dispense **200 µL** of well-mixed serum or plasma **sample** into the tube containing the reagent pellet.
6. 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.
7. Place the tube onto a laboratory mixer at medium speed and **incubate** at room temperature for **10 minutes**.
8. Loosen and remove the screw cap and insert the tube into the magnet until the collar of the tube contacts the magnet frame.
9. Magnetically separate the reagent for > 4 minutes to form a reagent pellet.
10. 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 (~ 200 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 9.
11. The sample is now ready for testing.

Enhanced Procedure

The VeraPrep Interference Enhanced procedure uses a 2:1 ratio of VeraPrep Interference reagent to serum or plasma sample (e.g., 400 µL reagent and 200 µL sample) to deplete biotin interference up to 400 ng/mL, anti-streptavidin and HAMA interference up to 40 µg/mL, and both anti-biotin antibody and HAAA interference up to 20 µg/mL. Smaller and larger sample volumes can be used if a 2:1 ratio of reagent: sample is maintained.

Enhanced Procedure Sample Volumes		
VeraPrep Interference (µL)	**Serum or Plasma (µL)**	**Samples (Uses per Vial)**
200	100	25
400	**200**	**12**
1,000	500	5

Example 2: VeraPrep Interference Enhanced Procedure:

1. Remove the VeraPrep Interference reagent vial from storage and vortex for a minimum of 10 seconds at medium speed to mix well and resuspend the reagent.
2. 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.
3. Dispense **400 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.
4. Carefully aspirate and discard all of the storage buffer supernatant (~400 µL) without disturbing the reagent pellet.
5. Dispense **200 µL** of well-mixed serum or plasma **sample** into the tube containing the reagent pellet.
6. 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.
7. Place the tube onto a laboratory mixer at medium speed and **incubate** at room temperature for **10 minutes**.
8. Loosen the screw cap and insert the tube into the magnet until the collar of the tube contacts the magnet frame.

9. Magnetically separate the reagent for > 4 minutes to form a reagent pellet.
10. 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 (~ 200 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 9.
11. The sample is now ready for testing.

PERFORMANCE CHARACTERISTICS

Biotin interference removal: A study was conducted on two different lots of reagent to demonstrate the ability of VeraPrep Interference to remove interfering biotin from serum samples. The study consisted of biotin-spiked serum and used the Immundiagnostik IDK® Biotin ELISA kit (Part No. K8141, measuring range of 48.1 – 1,200 pg/mL) to measure biotin levels before and after VeraPrep Interference treatment of the samples. The sample was spiked to 250 ng/mL biotin and the final concentration verified by LC-MS/MS (final concentration by LC-MS/MS is 248 ng/mL). The IDK® assay results were compared between untreated samples (Untreated) and VeraPrep Interference treated samples (Treated). In the presence of biotin greater than the IDK® assay measuring range the results were reported as > 1,200 pg/mL.

The results of the study indicated that the biotin depletion activity of the reagent was 0.2 ng of biotin per microliter of reagent.

The Untreated Sample was reported as >1,200 pg/mL. When 0.4 mL of 248 ng/mL biotin-spiked serum (total biotin of 99,200 picograms) was treated with 1.5 mg of reagent (500 uL of reagent) the Treated result was 661 pg/mL (or 264.4 picograms of biotin remaining), a total difference of 98,935 picograms. This depleted mass of biotin equals 0.2 ng of biotin depleted per microliter of reagent.

If using the Standard Procedure (e.g., a 1:2 ratio of reagent:sample such as 100 uL reagent used to treat 200 uL of sample) this will deplete a biotin concentration of 100 ng/mL in the sample to less than 1200 picograms/mL (< 1.2 ng/mL).

If using the Enhanced procedure (e.g., a 2:1 ratio of reagent:sample such as 400 uL reagent used to treat 200 uL of sample) this will deplete a biotin concentration of 400 ng/mL in the sample to less than 1200 picograms/mL (< 1.2 ng/mL).

Reagent neutrality: A study was conducted on two different lots of reagents to demonstrate the reagent neutrality of VeraPrep Interference, i.e., the ability of the reagent to treat samples and not affect analyte recovery in clinical assays. The study used the DRG PTH ELISA (DRG Part. No. EIA3645) assay as a model assay. A sample of known PTH concentration was tested both untreated and treated with VeraPrep Interference reagent. The results indicated less than a 10% variation in sample PTH concentration after treatment, demonstrating a lack of matrix effect in the assay, with an average treated result of 98.5% of the untreated concentration.

Heterophilic immunoglobulin interference removal: A study was conducted on two different lots of reagent to demonstrate the ability of VeraPrep Interference to remove up to 10 µg per mL reagent of anti-streptavidin interference, up to 10 µg/mL HAMA interference, up to 5 µg/mL anti-biotin antibody, and up to 5 ug/mL HAAA (goat, sheep, rabbit and bovine) interference using the Standard Procedure (1:2 ratio of reagent:sample). The study consisted of 6 samples (**Sample 1** = Anti-Streptavidin IgG, **Sample 2** = Anti-Mouse IgG, **Sample 3** = Anti-Goat IgG, **Sample 4** = Anti-Sheep IgG, **Sample 5** = Anti-Rabbit IgG, and **Sample 6** = Anti-Bovine IgG), and used SEC-HPLC (Agilent 1100 Series HPLC, Phenomenex BioSep SEC 3000 300 x 7.8mm column, 1.0 mL/min flow rate, and 50mM Potassium Phosphate, 250mM KCl, pH 6.8 mobile phase) to measure interfering IgG levels before and after VeraPrep Interference treatment of the samples. The SEC-HPLC peak area results of each interfering antibody sample were compared between untreated samples (Untreated) and VeraPrep Interference treated samples (Treated), and the % decrease

in Peak Area was used to determine the amount of interfering antibody removed from each sample. The results demonstrated a minimum removal of the following amounts of each interfering antibody:

- Anti-Streptavidin IgG: 0.027 µg IgG depleted per µL reagent
- Anti-Mouse IgG: 0.031 µg IgG depleted per µL reagent
- Anti-Goat IgG: 0.015 µg IgG depleted per µL reagent
- Anti-Sheep IgG: 0.012 µg IgG depleted per µL reagent
- Anti-Rabbit IgG: 0.015 µg IgG depleted per µL reagent
- Anti-Bovine IgG: 0.017 µg IgG depleted per µL reagent
- Anti-Biotin IgG: 0.009 µg IgG depleted per µL reagent

These results demonstrate VeraPrep Interference successfully removed up to 10 µg/mL anti-streptavidin interference (actual: 27 µg/mL), up to 10 µg/mL HAMA interference (31 µg/mL), up to 5 ug/mL anti-biotin antibody (9 ug/mL), and up to 5 µg/mL HAAA for anti-Goat (15 µg/mL), anti-Sheep (12 µg/mL), anti-Rabbit (15 µg/mL) and anti-Bovine (17 µg/mL) antibody interference.

As a negative control, the same method was conducted to measure non-specific, polyclonal Goat IgG levels before and after VeraPrep Interference treatment of the sample. VeraPrep Interference treatment resulted in a 0% decrease in Peak Area; i.e., did not deplete the non-specific Goat IgG.

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