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Datasheet for ABIN815816 HBd ELISA Kit

Overview

Quantity:	96 tests
Target:	HBd
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	0.007-30 ng/mL
Minimum Detection Limit:	0.007 ng/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of human hemoglobin subunit delta(HBD) concentrations in serum, plasma, lysate for RBC.
Sample Type:	Plasma, Red Blood Cell Lysate, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Human HBD.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	0.23 ng/mL
Components:	<ul style="list-style-type: none">Assay plate (12 × 8 coated Microwells)

Product Details

- Standard (freeze dried)
- Biotin-antibody (100 × concentrate)
- HRP-avidin (100 × concentrate)
- Biotin-antibody Diluent
- HRP-avidin Diluent
- Sample Diluent
- Wash Buffer (25 × concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip (for 96 wells)
- Instruction manual

Material not included:

- Microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 540nm or 570nm.
- An incubator which can provide stable incubation conditions up to 37°C ± 0.5°C.
- Squirt bottle, manifold dispenser or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100mL and 500mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

Target Details

Target:	HBd
Alternative Name:	HBD (HBd Products)
Background:	Synonyms: delta globin delta-globin chain
HGNC:	2766
UniProt:	P02042

Application Details

Application Notes:	<ul style="list-style-type: none">• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.• Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.• Grossly hemolyzed samples are not suitable for use in this assay.• If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products.
- Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Comment:

Detection wavelength: 450 nm

Information on standard material:

Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative.

Information on reagents:

In most cases the stop solution provided is 1 N H₂SO₄. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA.

Information on antibodies:

The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A

Sample Volume:

50 µL

Assay Time:

1 - 4.5 h

Plate:

Pre-coated

Protocol:

This assay employs the direct competitive inhibition enzyme immunoassay technique. Antibody

specific for HBD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with biotin-conjugated HBD. A competitive inhibition reaction is launched between HBD (Standards or samples) and biotin-conjugated HBD with the pre-coated HBD antibody. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in opposite to the amount of HBD bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagent Preparation:

- **Biotin-antibody (1×)** - Centrifuge the vial before opening.
Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent.
- **HRP-avidin (1×)** - Centrifuge the vial before opening.
HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent.
- **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×).
- **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s.
Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard. Sample Diluent serves as the zero standard (0ng/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL when pipetting.
- It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.

Sample Collection:

- **Serum:** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles.

- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2-8 °C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates:** Rinse 100 mg tissue with 1× PBS, homogenize in 1mL of 1× PBS and store overnight at -20 °C. After two freeze-thaw cycles to break the cell membranes, centrifuge the homogenates for 5 minutes at 5000 × g, 2-8 °C. Remove and assay the supernate immediately. Alternatively, aliquot and store samples at -20 °C or -80 °C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Calculation of Results:

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Precision:

Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision.

Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision.

- Intra-assay: CV% less than 8%
- Inter-assay: CV% less than 10%

Restrictions:

For Research Use only

Handling

Precaution of Use:

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.

Handling Advice:

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.

Handling

- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Storage:	4 °C/-20 °C
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Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
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Expiry Date:	6 months
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