Human Hemoglobin Beta ELISA Kit (Ready to Use)

Cat #: orb551008 (manual)

96 Tests

For research use only. Not intended for diagnostic use.

Product Features

Intend Use: The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of HBb in human serum, plasma or other biological fluids.

Sample Types: serum, plasma and other biological fluids

Detection Range: 1.56-100μg/mL

Sensitivity: 0.45μg/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of HBb.

Assay Principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to HBb. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific to HBb. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain HBb, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of HBb in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Kit Components

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	2
Standard	2	Standard Diluent	1×20mL
Detection Solution A	1×12mL	TMB Substrate	1×9mL
Detection Solution B	1×12mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Storage of the Kits

1. For unopened kits: All the reagents should be kept at -20℃ upon receipt.

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2. For opened kits: Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.

Note: All components are stable until this expiration date. It is highly recommended to use the remaining reagents within 1 month of opening.

Materials Required but Not Supplied

1. Microplate reader with 450 ± 10 nm filter.
2. Precision single or multi-channel pipettes and disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution.

Sample Preparation

1. Biorbyt is only responsible for the kit itself, 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.

2. 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. Samples should be diluted by diluent buffer included in the kit. If included diluent buffer is not enough, samples can also be diluted by 0.01 mol/L PBS (pH7.0-7.2).

3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

4. Tissue or cell extraction samples prepared using a chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.

5. Due to the possibility of mismatching between antigens from other origin and antibodies used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

6. Samples from cell culture supernatant may not be detected by the kit due to influence from factors such as cell viability, cell number and/or sampling time.

7. Fresh samples that have not been stored for extended periods of time are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and give inaccurate or incorrect results.

Sample Collection and Storage

● **Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 ºC before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquots at -20 ºC or -80 ºC for later use. Avoid repeated freeze/thaw cycles.

● **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 ºC within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquots at -20 ºC or -80 ºC for later use. Avoid repeated

freeze/thaw cycles.

● **Tissue homogenates** - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues should be rinsed in ice-cold PBS (0.01mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Mince the tissues into small pieces and homogenize them in 5-10 mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders also work). The resulting suspension should be sonicated with an ultrasonic cell disrupter or subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates are centrifugated for 5 minutes at 5000×g. Remove the supernate and assay immediately or aliquot and store at ≤-20℃.

● **Other biological fluids** - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquots at -20℃ or -80℃. Avoid repeated freeze/thaw cycles.

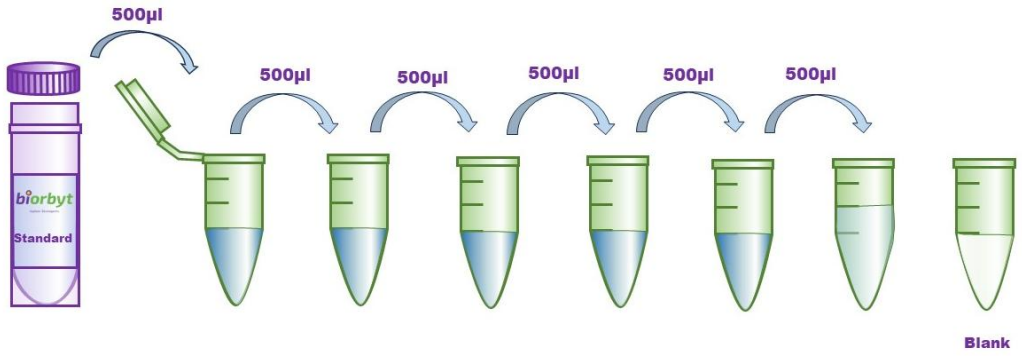
Note:

1. Samples to be used within 5 days may be stored at 4, otherwise samples must be stored at -20 ℃ (≤1 month) or -80 ℃ (≤2 months) to avoid loss of bioactivity and/or contamination.
2. Sample hemolysis will influence the result, and hemolytic specimen cannot be detected.
3. When performing the assay, bring samples to room temperature.

Reagent Preparation

1. **Bring all kit components and samples to room temperature (18-25°C) before use.**
2. **Standard** - Reconstitute the Standard with 1.0mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 100μg/mL. Prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Prepare a dilution series with 7 points; for example: 100μg/mL, 50μg/mL, 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.12μg/mL, 1.56μg/mL, and the last EP tube with Standard Diluent is the blank at 0μg/mL.

Stock Standard



Tube	1	2	3	4	5	6	7	8
ug/mL	100	50	25	12.5	6.25	3.12	1.56	0

3. **Detection Solution A and Detection Solution B** - Detection Solutions A and B are already at the correct concentrations and do not need to be diluted further.
4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips. Do not dump the residual solution back into the vial.

Note:

1. Do not perform a serial dilution directly in the wells.
2. Prepare standard within 15 minutes of performing the assay.
3. Carefully reconstitute Standards according to the instruction, avoid foaming and mix gently until the crystals are completely dissolved.
4. The reconstituted Standards can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Any contaminated water or container used during reagent preparation will influence the detection result.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for the standards, 1 well for blank. Add 100µL each of dilutions of standard (read Reagent Preparation), blank, and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 90 minutes at 37oC.
2. Remove the liquid from each well.
3. Add 100µL of Detection Solution A to each well. Incubate for 45 minutes at 37oC after covering it with the Plate sealer.
4. Aspirate the solution and wash with 300µL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by tapping the plate onto absorbent paper. Wash thoroughly 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100µL of Detection Solution B to each well. Incubate for 45 minutes at 37oC after covering it with the Plate sealer.
6. Repeat the aspiration/wash process for a total of 5 times as conducted in step 4.
7. Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-25 minutes at 37oC (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of the Substrate Solution.
8. Add 50µL of Stop Solution to each well. The liquid will turn yellow with the addition of the Stop solution. Mix the liquid by tapping the side of the plate. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

Note:

1. **Assay preparation:** Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remaining wells should be resealed and stored at -20°C.
2. **Samples or reagents addition: Please use the freshly prepared Standard.** Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards

and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. In addition, use separated reservoirs for each reagent.

3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods in between incubation steps. Once reagents are added to the well strips, DO NOT let the strips dry at any time during the assay. Incubation time and temperature must be controlled.

4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting, and remove any drops of water or fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.

5. **Controlling reaction time:** Observe the change of color after adding **TMB Substrate** (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in an inaccurate absorbance reading.

6. **TMB Substrate** is easily contaminated. Please protect it from light.

7. The environmental humidity may influence the results obtained from the kit. If the humidity in your facility is less than 60%, using a humidifier is recommended.

Assay Procedure Summary

1. Prepare all reagents, samples and standards
2. Add 100µL standard or sample to each well. Incubate 90 minutes at 37℃
3. Aspirate and add 100µL Detection Solution A. Incubate 45 minutes at 37℃
4. Aspirate and wash 3 times
5. Add 100µL Detection Solution B. Incubate 45 minutes at 37℃
6. Aspirate and wash 5 times
7. Add 90µL Substrate Solution. Incubate 15-25 minutes at 37℃
8. Add 50µL Stop Solution. Read at 450 nm immediately.

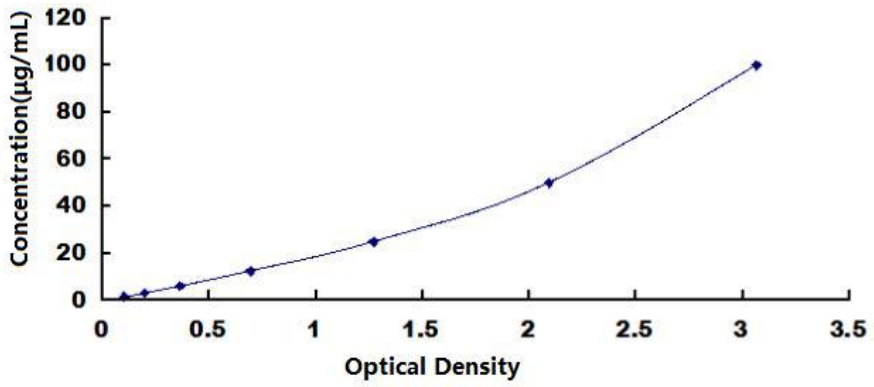
Calculation of Results

Average the duplicate readings for each standard, control and sample, then subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with HBb concentration on the y-axis and absorbance on the x-axis. Using plotting software, (for instance, curve expert 1.30), is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

To make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature

effects), plotting the log of the data to establish a standard curve for each test is recommended. The typical standard curve below is provided for reference only.



Typical Standard Curve for Human HBb ELISA.

Performance

Detection Range

1.56-100μg/mL. The standard curve concentrations used for the ELISA's were 100μg/mL, 50μg/mL, 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.12μg/mL, 1.56μg/mL.

Sensitivity

The minimum detectable dose of HBb is typically less than 0.45μg/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of HBb.

No significant cross-reactivity or interference between HBb and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible to perform all possible cross-reactivity detection tests between HBb and all analogues, therefore, cross reactivity may still exist.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level HBb were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level HBb were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage conditions.

Note:

To minimize unnecessary influences on the performance, operation procedures and lab conditions, especially room temperature, air humidity, and incubator temperatures should be strictly regulated. It is also strongly suggested that the whole assay is performed by the same experimenter from the beginning to the end.

ELISA Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample enzyme contaminated addition, the marker is and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.) and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.

	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions

The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

Declaration

1. Limited by the current conditions and scientific technology, it is impossible to conduct comprehensive identification and analysis tests on the raw materials provided by suppliers. As a result, it is possible there are some qualitative and/or technical risks.

2. The final experimental results will be closely related to the validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available to obtain accurate results.

3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction manual included in your kit. Electronic ones on our website are for reference only.

4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

5. Protect all reagents from strong light during storage and incubation. All bottle caps of reagents should be closed tightly to prevent evaporation of liquids and contamination by microorganisms.

6. There may be a foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.

7. Incorrect procedures during reagent preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10 nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.

8. Even the same experimenter may get different results from two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before the general assay for each batch is recommended.

9. Each kit has undergone several rigorous quality control tests. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance among kits from different batches could arise from the above factors as well.

10. Kits from different manufacturers with the same item might produce different results, since we have not compared our products with other manufacturers.

11. The standard in this kit, as well as the antigens used in antibody preparation are typically recombinant proteins. Differently expressed sequences, expression systems, and/or purification methods can be used in the preparation of recombinant proteins. There is also the possibility of differences in the screening technique of antibodies and antibody pairs in our kits. As a result, we cannot guarantee that our kit will be able to detect recombinant proteins produced by other companies. We do NOT recommend using Biorbyt ELISA kits for the detection of other recombinant proteins.

12. Validity period: 16 months.

13. The instruction manual also works with the 48T kit, but all reagents in the 48T kit are reduced by half.

Safety Notes

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this reagent.

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