ab157707 Hemoglobin Human ELISA Kit

For the quantitative measurement of Hemoglobin in Human serum and plasma samples.
This product is for research use only and is not intended for diagnostic use.

Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Human Hemoglobin ELISA Microplate	96 wells	4°C
Human Hemoglobin Calibrator (lyophilized)	1 vial	4°C
5X Diluent Concentrate	50 mL	4°C
20X Wash Buffer Concentrate	50 mL	4°C
Enzyme-Antibody Conjugate	150 µL	4°C
Chromogen Substrate Solution	12 mL	4°C
Stop Solution	12 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to perform this assay:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

1X Diluent Solution

The diluent solution is supplied as 5X Diluent Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O). The 1X Diluent Solution is stable for at least one week from the date of preparation and should be stored at 4°C.

1X Wash Buffer

The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30 - 35°C before dilution can dissolve crystals. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at room temperature (16 - 25°C) or at 4°C.

1X Enzyme-Antibody Conjugate

Calculate the required amount of 1X Enzyme-Antibody Conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

Chromogen Substrate Solution and Stop Solution

Ready to use as supplied.

Standard Preparation

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

1. Add 1.0 mL of distilled or de-ionized water to the Human Hemoglobin Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial.

Note: The reconstituted Human Hemoglobin Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles.

2. Label tube numbers 1 - 6.
3. Prepare **Standard #1** by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 150 µL of stock Human Hemoglobin Calibrator to obtain a concentration at 200 ng/mL and mix thoroughly and gently.

***Example:**

Note: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.

CS = Starting concentration of reconstituted Human Hemoglobin Calibrator
CF = Final concentration of Human Hemoglobin Calibrator for the assay procedure (200 ng/mL)
VA = Total volume of stock Human Hemoglobin Calibrator to dilute (e.g. 150 µL)
VD = Total volume of 1X Diluent Solution required to dilute stock Human Hemoglobin Calibrator to prepare **Standard #1**
VT = Total volume of **Standard #1**
DF = Dilution factor

Calculate the dilution factor (DF) between stock calibrator & the **Standard #1** final conc.:

$$Cs/CF = DF$$
$$897 / 200 = 4.485$$

Calculate the final volume VD required to prepare the **Standard #1** at 200 ng/mL

$$VA * DF = VT$$
$$VD = VT - VA$$
$$150 * 4.485 = 673\ \mu L$$
$$VD = 673 – 150 = 523\ \mu L$$

To tube #1, add 150 µL of reconstituted Human Hemoglobin Calibrator to 523 µL of 1X Diluent Solution to obtain a concentration at 200 ng/mL (**Standard #1**).

4. Add 300 µL 1X Diluent Solution into tube numbers 2 - 6.
5. Prepare **Standard #2** by adding 300 µL Standard #1 to tube #2. Mix thoroughly and gently.
6. Prepare **Standard #3** by adding 300 µL from **Standard #2** to #3. Mix thoroughly and gently.
7. Using the table below as a guide to prepare further serial dilutions. 1X Diluent Solution serves as the zero standard (0 ng/mL)

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc (ng/mL)	Final Conc. (ng/mL)
1	See step 3				200
2	300	300	600	200	100
3	300	300	600	100	50
4	300	300	600	50	25
5	300	300	600	25	12.5
6	300	300	600	12.5	6.25

Sample Collection and Storage

Serum: Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

Plasma: For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results.

Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Sample Preparation

General Sample information:

The assay for quantification of Hemoglobin in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/1,000 is appropriate for most samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one/two representative samples before running the entire plate is recommended.

- To prepare a 1/1,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution.
- Next, dilute the 1/100 samples by transferring 25 µL, to 225 µL of 1X diluent. You now have a 1/1,000 dilution of your sample.
- Mix thoroughly at each stage.

Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

1. Pipette 100 µL of each standard, including zero control, in duplicate, into pre designated wells.
2. Pipette 100 µL of sample (in duplicate) into pre designated wells.
3. Incubate the microtiter plate at room temperature for twenty (20 ± 2) minutes. Keep plate covered and level during incubation.
4. Following incubation, aspirate the contents of the wells.
5. Completely fill each well with appropriately diluted 1X Wash Buffer and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
6. Pipette 100 µL of appropriately 1X Enzyme-Antibody Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.
7. Wash and blot the wells as described.
8. Pipette 100 µL of TMB Substrate Solution into each well.
9. Incubate in the dark at room temperature for precisely ten (10) minutes.
10. After ten minutes, add 100 µL of Stop Solution to each well.
11. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

Calculations

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four-parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation Buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

Typical Sample Values

Sensitivity – Calculated minimum detectable dose =0.8445 ng/mL

Precision – Both intra and Inter assay Precision is at <10% CV

Recovery – Control Serum Recovery = > 85%

Interferences - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Please contact our Technical Support team for more information.

Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve powder thoroughly by mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)