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HUABIO RESEARCH INC

Human Hemoglobin subunit alpha/HBA1 ELISA Instructions Cat:EH0247

Content

	CAT	Volume
❶ CP (Coated Plate)	EH0247CP	96 well
❷ S (Standard)	EH0247S	2 vial
❸ SD (Sample Diluent)	ESD21	15 ml/bottle
❹ DA (Detect Antibody)	EH0247DA	6 ml/bottle
❺ SH (Streptavidin-HRP)	ESH01	12 ml/bottle
❻ AB (Assay Buffer 1×)	EAB01	12 ml/bottle
❼ TS (TMB Substrate)	ETS01	12 ml/bottle
❽ SS (Stop Solution)	ESS01	12 ml/bottle
❾ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❿ SF (Sealer Film)	ESF01	6 pieces

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

Sample Dilution

Samples such as serum、plasma require at least a 20-fold dilution into Sample Diluent. it is recommended to add 10 µl of sample + 190 µl of Sample Diluent.

REAGENT PREPARATION

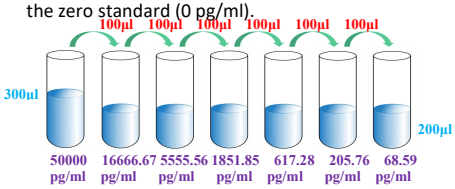
Standard Curve Preparation:

The **SD** (Sample Diluent) is used for serum and plasma. Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. Urine sample use AB (Assay Buffer) prepare standard curve.

Reconstitute human Hemoglobin subunit alpha/HBA1 Standard by addition of distilled water as S. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 500000pg/ml).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The human Hemoglobin subunit alpha/HBA1 Standard EH0247S 500000pg/ml 30 µl + 270 µl SPB serves as the high standard (50000pg/ml). Pipette 200 µl of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).



Washing Buffer (1×) Preparation

Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 µl of **AB** (Assay Buffer) to each well.

❹ Add 50 µl or 10 µl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 µl of **DA** (Detect Antibody) to each well.

❻ Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hour on a microplate **shaker** set at 500 rpm.

❼ Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.

❽ Add 100 µl of **SH** (Streptavidin-HRP) to each well.

❾ Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.

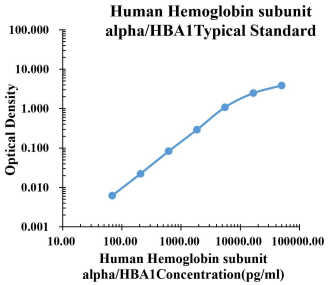
❿ Repeat aspiration/**wash** as in step 7.

⓫ Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

⓬ Add 100 µl of **SS** (Stop Solution) to each well.

⓭ Determine the optical density within 30

TYPICAL DATA



pg/ml	O.D.		Average	Corrected
0.00	0.0079	0.0078	0.0079	
68.59	0.0139	0.0142	0.0141	0.0062
205.76	0.0293	0.0308	0.0301	0.0222
617.28	0.0938	0.0880	0.0909	0.0831
1851.85	0.3062	0.2938	0.3000	0.2922
5555.56	1.0950	1.1060	1.1005	1.0927
16666.67	2.5210	2.4460	2.4835	2.4757
50000.00	3.9190	3.8470	3.8830	3.8752

SENSITIVITY

The minimum detectable dose (MDD) of human Hemoglobin subunit alpha/HBA1 is typically less than 9.11 pg/ml (50 µl of sample volume) or 28.56 pg/ml (10 µl of sample volume).

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the

PRECISION

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

Inter-assay Precision (Precision between assays)

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (pg/ml)	945.9	4900.6	14097.9	1278.0	5808.4	16647.0
Standard deviation	72.9	355.0	1048.4	88.4	144.2	638.3
Coefficient of variation (%)	7.7	7.2	7.4	6.9	2.5	3.8

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human Hemoglobin subunit alpha/HBA1 into health human serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 94% to 122% with an overall mean recovery of 104%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of Hemoglobin subunit alpha/HBA1 in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 97% to 110% with an overall mean recovery of 103%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of human Hemoglobin subunit alpha/HBA1 in this assay. No medical histories were available for the

Sample Matrix	Sample Evaluated	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	78.22-476.01	100	193.92

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.