ab326009 – Human alpha Fetoprotein SimpleStep ELISA® Kit (AFP), Chemiluminescent

For the quantitative measurement of alpha Fetoprotein in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and urine.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326009

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve, native serum and plasma (citrate) linearity, and sensitivity were collected using the colorimetric version of this kit (ab193765).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human alpha Fetoprotein Lyophilized Capture Antibody	1 Vial	10 x 1 Vial	+4°C
Human alpha Fetoprotein Lyophilized Detector Antibody	1 Vial	10 x 1 Vial	+4°C
Human alpha Fetoprotein Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 5BI	6 mL	10 x 6 mL	+4°C
Sample Diluent 75BS	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	12 mL	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent 75BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

10X Capture Antibody: To reconstitute the lyophilized capture antibody, centrifuge at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

10X Detector Antibody: To reconstitute the lyophilized detector antibody, centrifuge at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the alpha Fetoprotein standard sample by adding the volume of Sample Diluent 75BS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 80,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 200 µL of Sample Diluent 75BS into tube number 1 and 150 µL of Sample Diluent 75BS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	200	200	80,000	40,000
2	Standard#1	75	150	40,000	13,333.3
3	Standard#2	75	150	13,333.3	4,444.4
4	Standard#3	75	150	4,444.4	1,481.5
5	Standard#4	75	150	1,481.5	493.8
6	Standard#5	75	150	493.8	164.6
7	Standard#6	75	150	164.6	54.9
8	Standard#7	75	150	54.9	18.3
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	≤100%
Plasma – Heparin	≤100%
Plasma – EDTA	≤100%
Plasma – Citrate	≤100%
Urine*	≤100%
HepG2 Cell Culture Supernatant	1:10,656 - 1:666
Pregnant Human Serum	1:533 - 1:33

*Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Assay, or dilute samples into Sample Diluent 75BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA, or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples into Sample Diluent 75BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent 75BS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent 75BS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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).
Differences in well absorbance or "edge effects" have not been observed with this assay.

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. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

Version 2 | 2026-03-16

For all technical or commercial enquiries please go to:
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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human alpha Fetoprotein.

The standard protein in this kit is full length human alpha Fetoprotein.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant sample types.

Neat pooled serum and plasma (citrate, EDTA, heparin,) samples from apparently healthy donors were measured in duplicate. The mean AFP concentration was determined to be 335 pg/mL in serum, 215 pg/mL in plasma (citrate), 315 pg/mL in plasma (EDTA), 504 pg/mL in plasma (heparin).

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), and urine sample types.

Saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

SPECIES REACTIVITY
10 ng/mL of recombinant mouse AFP was tested for cross reactivity. No cross reactivity was observed.

Other species reactivity not determined.

CALIBRATION
This immunoassay is calibrated against a highly purified human alpha Fetoprotein. The NIBSC/WHO unclassified purified human alpha Fetoprotein preparation (NIBSC code AFP) was evaluated in this kit.

The dose response curve of the unclassified standard alpha Fetoprotein parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human alpha Fetoprotein kit to approximate NIBSC units, use the equation below.

NIBSC (AFP) approximate value (IU/mL) = 0.927 x SimpleStep Human alpha Fetoprotein value (ng/mL).

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,693	1,665	1,679
18.3	3,171	2,997	3,084
54.9	8,707	7,281	7,994
164.6	24,133	23,506	23,820
493.8	59,801	65,606	62,704
1,481.5	183,280	195,220	189,250
4,444.4	581,320	596,690	589,005
13,333.3	1,692,600	1,646,100	1,669,350
40,000	5,196,200	5,239,200	5,217,700

Table 1. Example of human alpha Fetoprotein standard curve in Sample Diluent 75BS. The alpha Fetoprotein standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES
Sensitivity:
The calculated minimal detectable dose (MDD) is 13.9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery
Three concentrations of alpha Fetoprotein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Neat Serum	91	88 - 96
Neat Plasma – Citrate	89	89 - 89

Neat Plasma – EDTA	100	98 - 102
Neat Plasma – Heparin	96	94 - 97
Neat Urine	119	116 - 121
Neat Cell Culture Media*	94	91 - 98
1% Pregnant Serum	104	88 - 132
1: 2,000 HepG2 Cell Culture Supernatant	103	101 - 113

*Media is RPMI 1640 containing 10% fetal bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native alpha Fetoprotein was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 75BS.

Dilution Factor	Interpolated value	3% Pregnant Human Serum	1:666 HepG2 Supernatant	100% Human Serum*	100% Human Plasma (Citrate)*
Undiluted	pg/mL	6,387	8,532	314.7	314.6
	% Expected value	100	100	100	100
2	pg/mL	3,423	4,602	182.7	146.1
	% Expected value	107	108	116	93
4	pg/mL	1,669	2,335	90.4	66.7
	% Expected value	104	109	115	85
8	pg/mL	872.2	1,199	43.9	33.4
	% Expected value	109	112	112	85
16	pg/mL	399.2	598.1	18.5	ND
	% Expected value	100	112	94	ND

ND – Not Detected – below product dynamic range
*Data collected using Human alpha Fetoprotein SimpleStep ELISA® Kit, Chemiluminescent.

Recombinant alpha Fetoprotein was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 75BS.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)	100% Human Plasma (Heparin)	100% Urine
Undiluted	pg/mL	6,815	7,156	7,552	7,635	8,301
	% Expected value	100	100	100	100	100
2	pg/mL	3,975	3,954	3,883	3,942	4,232
	% Expected value	117	111	103	103	102
4	pg/mL	1,915	1,905	1,901	1,834	1,963
	% Expected value	112	106	101	96	95
8	pg/mL	976.9	899.0	845.8	951.4	1010
	% Expected value	115	101	90	100	97
16	pg/mL	478.0	389.2	420.0	482.9	496.8
	% Expected value	112	87	89	101	96

Precision

Mean coefficient of variations of interpolated values of alpha Fetoprotein from two concentrations of pregnant human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.0	4.5

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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