ab108680

alpha Fetoprotein
Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human alpha Fetoprotein concentration in serum and plasma.

This product is for research use only and is not intended for in vitro diagnostic use.

www.abcam.com
ab108680 alpha Fetoprotein Human ELISA Kit
<table>
<thead>
<tr>
<th></th>
<th>Table of Contents</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Assay Summary</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Kit Contents</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Storage and Handling</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Additional Materials Required</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Preparation of Reagents</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Preparation and Collection of Specimen</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Assay Method</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>Data Analysis</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>Limitations</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>Specificity</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>Troubleshooting</td>
<td>16</td>
</tr>
</tbody>
</table>
ab108680 alpha Fetoprotein Human ELISA Kit

1. Introduction

ab108680 alpha Fetoprotein Human ELISA Kit is an immunoenzymatic colorimetric method for quantitative determination of alpha Fetoprotein in human serum and plasma.

Alpha Fetoprotein (AFP) is a 68 kDa glycoprotein, which is normally only produced in the fetus during its development. It is normally produced by the liver and yolk sac of the fetus. AFP levels decrease soon after birth and probably has no function in normal adults. It binds the hormone estradiol to keep it from affecting the fetal brain. Its measurement during pregnancy has been useful to detect certain abnormalities - specifically, if high levels of AFP are found in amniotic fluid, it can indicate a developmental defect in the baby. In some patients who are not pregnant a tumor can produce AFP, thus it can be used as a tumor marker. AFP is the main tumor marker (along with HCG) to diagnose testicular cancer and its values over time can have significant effect on the treatment plan.
ab108680 is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system is based on simultaneous binding of human AFP to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP).

Microtiter strip wells are precoated with anti-AFP IgG antibodies. AFP in samples and standards binds to the immobilised antibodies on the surface of the microtiter wells and the second, soluble anti-AFP antibody-enzyme conjugate binds to the immobile antibody-AFP-complex during the first incubation.

Afterwards a bound/free separation is performed by solid-phase washing.

The immune complex is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of AFP in samples and standards.

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorption at 450 nm is read using an ELISA microwell plate reader.
3. Kit Contents

- **Coated Wells**: 12 break apart 8-well snap-off strips coated with anti-AFP IgG, in resealable aluminium foil.

- **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).

- **Anti-AFP-HRP conjugate conc.**: 1 bottle containing min. 0.4 ml of horseradish peroxidase labeled anti-AFP antibodies.

- **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3’, 5, 5´-tetramethylbenzidine (H₂O₂-TMB 0.26g/l) (avoid any skin contact).

- **Incubation buffer**: 1 bottle containing 50 ml phosphate buffer 50 mM, pH 7.4, BSA 1 g/l.

- **Wash solution 50x conc.**: 1 bottle containing 20 ml (NaCl 45 g/l, Tween20 55 g/l).

- **AFP Standards**: 5 bottles, 1 ml each: The standards are calibrated against the (WHO 2nd IRP 72/225) and have approximately the following concentrations:
  
<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0ng/ml</td>
</tr>
<tr>
<td>1</td>
<td>5ng/ml</td>
</tr>
<tr>
<td>2</td>
<td>20ng/ml</td>
</tr>
<tr>
<td>3</td>
<td>80ng/ml</td>
</tr>
<tr>
<td>4</td>
<td>200ng/ml</td>
</tr>
</tbody>
</table>

- **Strip holder**: 1

- **Cover foil**: 1
4. Storage and Handling

The reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C. After first use the standard solutions are still stable for another 6 months if stored at 2 - 8 °C.

5. Additional Materials Required

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 µl and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

6. Preparation of Reagents

It is very important to bring all reagents, samples and standards to room temperature (22 - 28°C) before starting the test run.

1. **Coated snap-off Strips:** The ready to use break apart snap-off strips are coated with anti-AFP IgG antibodies. Store at 2 - 8°C.
Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8 °C. Do not remove the adhesive sheets on the unused strips.

2. **Anti-AFP-HRP Conjugate:** The bottle contains min 0.4 ml of a concentrated solution with anti-AFP antibodies conjugated with horseradish peroxidase. Dilute immediately before use: Add 10 µl concentrated conjugate to 1.0 ml of Incubation Buffer. Mix gently for 5 min with a rotating mixer. Stable for 3 hours at room temperature.

3. **AFP Standards:** Each of the 5 vials contains 1 ml standard solution of the concentration mentioned in Kit Contents. The standards are ready to use. *After first use the standard solutions are still stable for another 6 months if stored at 2 - 8 °C.*

4. **TMB Substrate Solution:** The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2 - 8 °C.*

5. **Stop Solution:** The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2 – 8 °C. *After first use stable until expiry date.*
6. **Wash Solution:** Dilute the contents of the concentrated wash solution (50x) to 1 l with distilled or deionized water in a suitable storage container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days.

7. **Preparation and Collection of Specimen**

Use human serum or plasma samples with this assay. If the assay is performed within 48 hours after sample collection, the specimens should be kept at 2 - 8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Samples with concentration above 200 ng/ml should be diluted 1/1 with Incubation buffer.

**Precaution:**

- The reagents contain Proclin 300® as preservative.
- Do not use heavily haemolysed samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- This method allows the determination of AFP from 5 ng/ml to 200 ng/ml.
8. Assay Method

Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) for standard 0
2 wells (e.g. D1+E1) for standard 1
2 wells (e.g. F1+G1) for standard 2
2 wells (e.g. H1+A2) for standard 3
2 wells (e.g. B2+C2) for standard 4

It is recommended to determine standards and samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each standard and each sample.
Assay Procedure:

1. Dispense 25 µl standards and samples into their respective wells. Add 50 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour at room temperature (22 – 28 °C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for exactly 15 min at room temperature (22 – 28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
   *Any blue color developed during the incubation turns into yellow.*
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.
Quality control:

Each laboratory should assay controls at normal, high and low levels range of AFP for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

9. Data Analysis

1. Calculate the mean absorbance (Em) for each point of the standard curve and each sample.
2. Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples.
3. Plot the mean value of absorbance of the standards (Em) against concentration.
4. Draw the best-fit curve through the plotted points. (e.g. Four Parameter Logistic).
5. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

A. Reference Values

In a study conducted with apparently normal healthy adults, using AFP, the following results were observed:

<table>
<thead>
<tr>
<th>Population</th>
<th>0-10 ng/ml</th>
<th>20 ng/ml</th>
<th>30 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>82</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>55</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

In a study conducted with nonseminomatous testicular cancer patient using AFP, the following values were observed:

<table>
<thead>
<tr>
<th>Population</th>
<th>0-10 ng/ml</th>
<th>10-100 ng/ml</th>
<th>&gt;100 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
B. Sensitivity

The lowest detectable concentration of AFP that can be distinguished from the standard 0 is 1.0 ng/ml at the 95 % confidence limit.

C. Hook Effect

ab108680 alpha Fetoprotein Human ELISA Kit, a competitive enzyme immunoassay, shows no Hook Effect up to 4000 ng/ml.

D. Reproducibility

**Intra-Assay:** Within-run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6.6%.

**Inter-Assay:** Between-run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 6.3%.

E. Recovery

The recovery of 12.5 – 25 – 50 – 100 ng/ml of AFP added to sample gave an average value (±SD) of 99.19 % ± 4.67 % with reference to the original concentrations.
10. Limitations

- Sample(s), which are contaminated microbiologically, should not be used in the assay.
- Highly lipemic or haemolysed specimen(s) should similarly not be used.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
11. Specificity

The cross-reactivity values of ab108680 alpha Fetoprotein Human ELISA Kit has been calculated on a weight/weight basis:

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>100.0 %</td>
</tr>
<tr>
<td>b-HCG</td>
<td>0.01 %</td>
</tr>
<tr>
<td>HCG</td>
<td>0.01 %</td>
</tr>
<tr>
<td>LH</td>
<td>0.01 %</td>
</tr>
<tr>
<td>FSH</td>
<td>0.01 %</td>
</tr>
<tr>
<td>TSH</td>
<td>0.01 %</td>
</tr>
</tbody>
</table>
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn’t fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Issue</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).
ab108680 alpha Fetoprotein Human ELISA Kit
**Abcam in the USA**
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge, MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

**Abcam in Japan**
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

**Abcam in Europe**
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

**Abcam in Hong Kong**
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888

Copyright © 2011 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.