Cat. # MK151

For Research Use

TaKaRa

Human Alpha Fetoprotein (AFP) EIA Kit

Product Manual

v201607Da

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I. Description

The liver has well-known roles in detoxification and sugar and fat metabolism. In addition, it has been recently discovered that the liver plays an important role in hematopoiesis, in particular in maintenance and preservation of hematopoietic stem cells and the production of blood cells during the early development of bone marrow. During early mouse fetal development (starting at approximately embryonic day 9), alpha-fetoprotein (AFP), an early marker of undifferentiated cells, is expressed in a hepatocyte-specific manner. AFP is a 70 kDa glycoprotein (590 amino acids) that is produced in the liver and yolk sac during the fetal development. AFP produced during fetal development is thought to be important for hematopoietic function.

Because albumin replaces AFP function after birth, AFP is rarely detected in normal adult blood. However, AFP concentration in the blood increases during acute hepatitis, cirrhosis of the liver, and primary liver cancer. Thus, AFP may be a useful marker for early detection and differential diagnosis of hepatic disorders, along with CA19-9 (a glycan tumor marker that is extremely useful in the detection of pancreatic cancer and bile duct cancer) and CEA (carcinoembryonic antigen, a marker of cancer of the digestive system).

Recently, it has become possible to efficiently differentiate pluripotent stem cells (iPS cells and ES cells) into various cell and tissue types. During liver cell differentiation from pluripotent cells, parallel measurement of AFP and albumin allows the stage of differentiation and the acquisition of differentiated cellular function to be monitored.

The Human Alpha Fetoprotein (AFP) EIA Kit is an assay kit that uses monoclonal antibodies for specific and high-sensitivity measurement of AFP in serum or cell culture supernatant. The antibodies in this kit do not cross-react with bovine antigens. Therefore, accurate measurement can be obtained even in the presence of fetal bovine serum. This kit can detect even low-levels of AFP secreted in culture supernatant during the early phases of cellular differentiation.

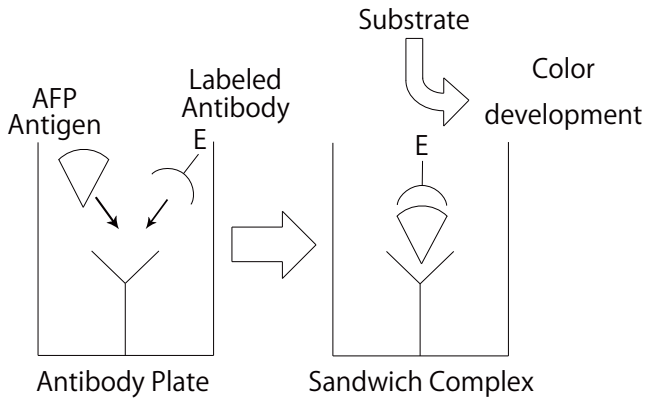


Figure 1. Principle of sandwich ELISA

II. Components

(1) Antibody Coated Microtiter Plate Anti-Human AFP Monoclonal Antibody Coated Plate (96 wells: 8 wells x 12 strips)	1 plate
(2) Antibody-POD Conjugate (lyophilized) Peroxidase-labeled Anti-Human AFP Monoclonal Antibody	for 11 ml
(3) Standard (lyophilized) Purified Human AFP (purified from umbilical cord blood) 160 ng	for 1 ml
(4) Sample Diluent BlockAce Powder in PBS (contains a preservative)	11 ml x 2
(5) Substrate Solution (TMBZ) 3,3', 5,5'- Tetramethylbenzidine solution	12 ml

III. Materials Required but not Provided

- Wash and Stop solution for ELISA without Sulfuric Acid (Cat. #MK021)
  Contains wash solution (10X PBS, 50 ml x 5 tubes; Tween 20, 3 ml) and reaction stop solution (60 ml).
  * This product is a stop solution for peroxidase reactions without 1N sulfuric acid.
  * 1N sulfuric acid can be used as a stop solution. Handle 1N sulfuric acid with caution.
- Pipette, micropipette, and tips
- Microplate reader (capable of measuring absorbance of up to 3.5 when set to 450 nm)

IV. Storage 4℃

V. Intended Use

- The measurement of alpha-fetoprotein level in human serum samples.
- Monitoring cell differentiation by measurement of human alpha-fetoprotein in cell culture supernatant during liver cell differentiation.
- This kit can also be used to measure porcine alpha-fetoprotein.

Caution: This kit is for research use. It is not intended for diagnostic purposes.

VI. Protocol

1. Samples

- Store samples at 2 - 10℃ or freeze if measurement will not be performed for 12 hours or more.
- Dilution of specimens must be investigated in advance. When high levels of AFP are expected, dilute samples using (4) Sample Diluent.
- In general, normal serum samples can be diluted approximately 2 times.
- Because this kit utilizes a 1-step method, it is recommended that serum is used as the sample for measurement. Anticoagulants present in plasma may affect the labeling antibody in this assay.
- Because AFP level in culture supernatant of differentiating cells differs greatly according to the stage of differentiation and the number of cells, it is necessary to perform preliminary studies to determine the optimal sample dilution ratio.
- Because the antibodies in this kit do not cross-react with bovine antigens, it is possible to perform direct measurement of cell culture supernatant without being affected by serum-containing culture medium.

2. Reagent Preparation

- Return the antibody plate ((1) Antibody Coated Microtiter Plate) to room temperature and open it before use.
- Labeled Antibody Solution
  Dissolve (2) Antibody-POD Conjugate with 11 ml of distilled water.
  The solution is stable for 1 week at 4℃. Store frozen at -20℃ if storing for longer than 1 week. The frozen solution is stable for 1 month at -20℃. Once thawed, do not refreeze.
- Human AFP Standard Solution
  Add 1 ml of distilled water to (3) Standard and dissolve completely to prepare the human AFP standard solution (160 ng/ml). To prepare standards, dilute the Standard with (4) Sample Diluent and prepare solutions with concentrations of 80, 40, 20, 10, 5, and 2.5 ng/ml. Use (4) Sample Diluent alone as the 0 ng/ml concentration. Dissolved Human AFP standard solution (160 ng/ml) is stable for 1 week when stored at 4℃, and for 1 month when stored at -20℃.
- (5) Substrate Solution (TMBZ)
  Bring (5) Substrate Solution (TMBZ) to room temperature before use. It is supplied ready to use. Check before use that the Substrate Solution has not developed a dark blue color. Reaction with metal ions will result in this coloration; make sure it is not contaminated with any tap water.
  If the Substrate Solution will be used for several assays, aliquot the required volume in advance.
- Stop Solution
  Use the stop solution in the Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021).
  ＊ Because this is highly viscous, mix well using a plate mixer after its introduction.
- PBS for Wash
  Dilute 10X PBS in the Wash and Stop Solution for ELISA without Sulfuric Acid with distilled water to obtain a 1X solution.
  96 reactions requires approximately 300 ml of wash solution.

3. Procedure

Assay samples in duplicate. Allow reagents in the kit and samples to return to room temperature and make sure that solutions are mixed uniformly without creating bubbles before use.

Note: Prepare reagents and samples in a separate 96 well plate in advance so that they can be added to the antibody-plate quickly (within 5 minutes) using an 8-channel pipette or similar apparatus.

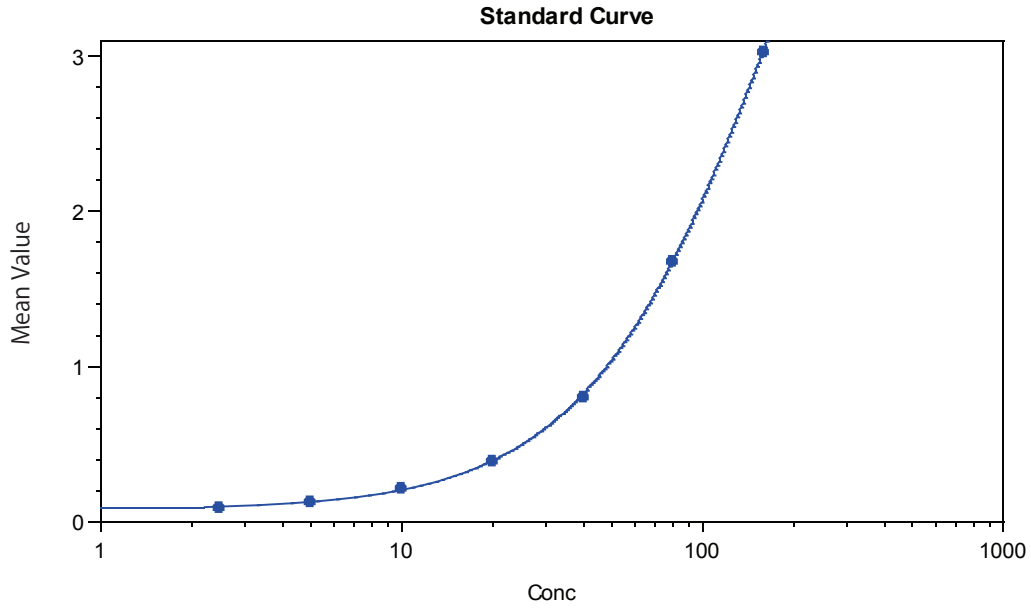
1. Add 100 µl Labeled Antibody Solution to each well of the plate. Next, add 50 µl of each test sample and Human AFP Standard Solutions (perform duplicate measurements) that were prepared previously to the wells and mix for 5 seconds using a plate mixer. Wrap with film to prevent evaporation of the solutions. To obtain highly reliable results, it is recommended to place serial dilutions of the Standard Solution (100 µl/well) in the 1st and 12th rows. Perform the reaction for 1 hour at room temperature (20 - 30℃); incubation at 37℃ may compromise antigenicity. [First reaction]
2. Remove the reaction mixture from wells, discarding the liquid. Wash wells 5 times with PBS. Remove excess PBS and then add 100 µl of (5) Substrate Solution (TMBZ) per well using an 8-channel pipette and allow to react for 15 minutes at room temperature (20 - 30℃). [Second reaction]
3. Add 100 µl of Stop Solution to each well in the same order that the (5) Substrate Solution (TMBZ) was added and mix well after stopping the reaction.
4. To make a zero adjustment, use distilled water as a blank and measure absorbance at 450 nm. The color is stable for up to 1 hour after reaction termination.
5. Prepare the standard curve by plotting the concentration of each standard solution on the horizontal axis and the corresponding absorbance on the vertical axis. Use the standard curve to determine the corresponding concentration of human AFP from the absorbance of the samples.

VII. Performance

1. Standard curve (Human Alpha Fetoprotein EIA Kit)

The standard curve below is a typical example. Please prepare a standard curve for each measurement to obtain accurate results.

Limit of detection: 2.5 ng/ml
Curve Fit: 4-Parameter



4-P Fit : $y = (A - D)/(1 + (x/C)^B) + D$:

A	B	C	D	R^2
0.0703	1.35	186	6.64	1

AFP (ng/ml)	160	80	40	20	10	5	2.5	0
A_{450}	3.030	1.680	0.796	0.386	0.210	0.126	0.089	0.060

Color Development: 15 minutes

2. Reproducibility

<Intra-assay precision test (n=8)>

A reproducibility test was performed using three concentrations of culture supernatant derived from AFP-secreting cells as the control samples.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	CV (%)
Control A	66.9	6.489	9.7
Control B	31.3	1.252	4.0
Control C	12.8	0.704	5.5

<Inter-assay precision test (n=3)>

A reproducibility test was performed by assaying three concentrations of controls over 3 days.

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	CV (%)
Control A	64.2	1.926	3.0
Control B	29.8	1.788	6.0
Control C	11.6	1.021	8.8

3. Recovery Test

Equal volumes of samples in different concentrations were combined and the recovery rate was investigated using the anticipated theoretical values and the measured values.

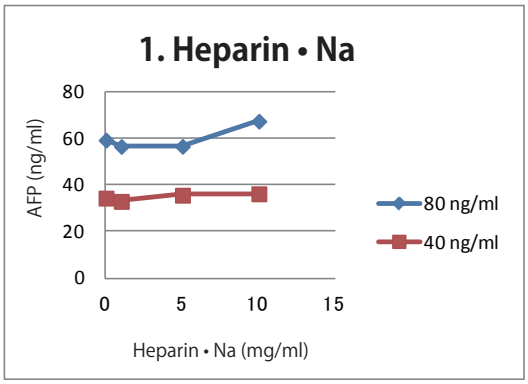
Sample A	Sample B	Theoretical Value (A+B)/2	Assay Result	Recovery Rate (%)
63.21	130.7	96.98	113.89	117.4
63.21	64.2	63.71	61.57	96.6
63.21	32.1	47.64	42.66	89.6
63.21	16.7	39.95	36.03	90.2
63.21	11.6	37.41	31.03	82.9
25.98	130.7	78.36	79.12	100.1
25.98	64.2	45.09	46.42	102.9
25.98	32.1	29.02	39.90	137.5
25.98	16.7	21.33	26.51	124.3
25.98	11.6	18.79	18.33	97.5
8.92	130.7	69.83	74.10	106.1
8.92	64.2	36.56	39.35	107.6
8.92	32.1	20.49	21.15	103.2
8.92	16.7	12.81	14.75	115.2
8.92	11.6	10.27	10.45	101.8

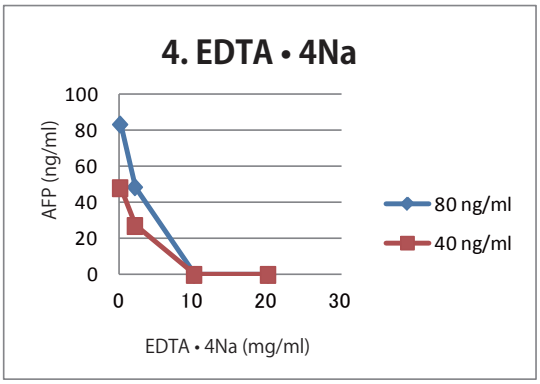
Units: ng/ml

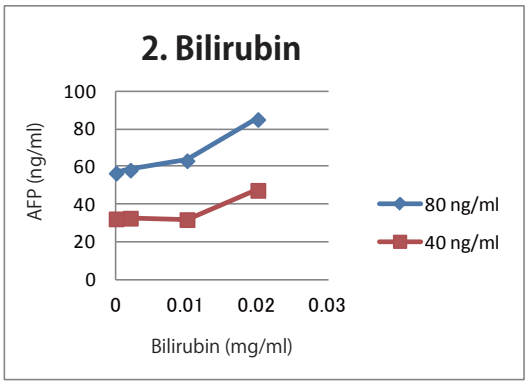
4. Effect of Coexisting Materials

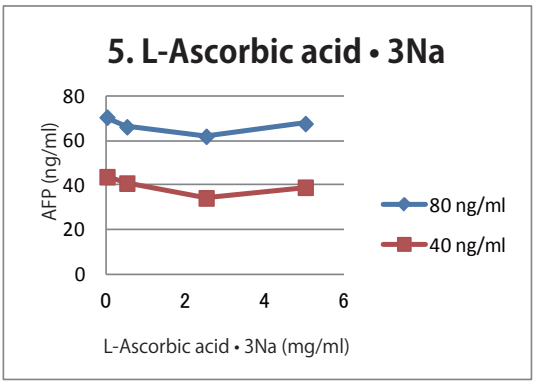
Each sample was combined with a test material at a ratio of 9 to 1 to investigate the effect on the reaction system.
The concentrations of the test materials shown in the graphs are the final concentration.

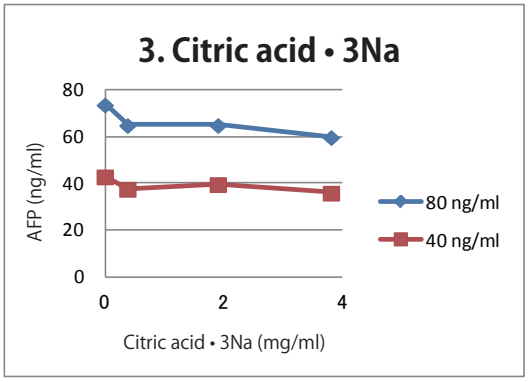
Caution: EDTA • 4Na chelating agent inhibited measurement; use of EDTA • 4Na as an anticoagulant is not recommended.

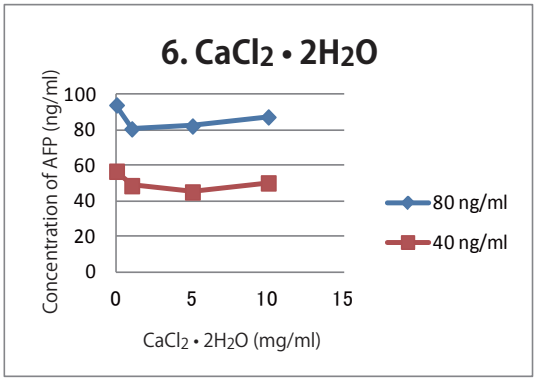












5. Effects of Freezing-Thawing on Samples

The effect of freezing-thawing samples on AFP measurement was investigated. Samples that were frozen and thawed (-80 to 25℃) 8 times consecutively and samples that were thawed only once were assayed simultaneously.

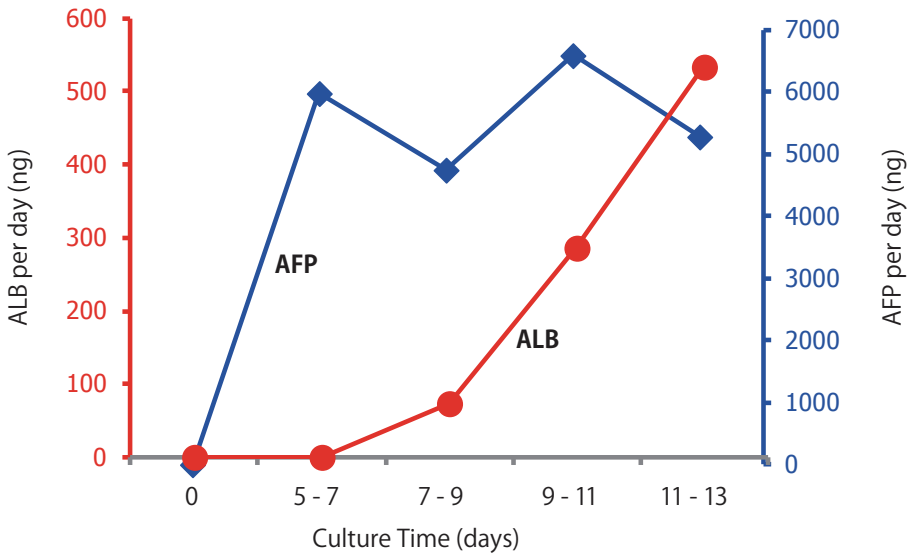
	After 8 Freeze-Thaw Cycles	After 1 Freeze-Thaw Cycle
Sample A	64.292	69.708
Sample B	33.599	31.304
Sample C	12.908	12.250

(Units: ng/ml)

Results: No change in measured AFP concentration was observed after 8 freeze-thaw cycles.

6. Example of Measurement

Human AFP and human albumin (ALB) secreted in cell culture media during differentiation of pluripotent cells into liver cells was monitored. Measurement of albumin was performed using the Human Albumin EIA Kit (Cat. #MK132).
In the graph, the time of culture is plotted on the x-axis and the amount of protein (ng) produced per ml of culture medium is plotted on the y-axis.



Results: Secretion of alpha-fetoprotein (AFP) and albumin (ALB) into the culture supernatant was observed during hepatic cell differentiation.

7. Cross-Reactivity with Various Animal Serums

AFP levels in serum from various animals was measured using this kit.

Animal	Cross-Reactivity
Adult Pig	below limit of detection
Micro Mini Pig, 6 Months of Age	below limit of detection
Micro Mini Pig, 10 Days of Age	◎ cross-reaction Serum concentration ~ 2,000 ng/ml
Fetal Bovine Serum	× no cross-reaction

Results: Clear cross-reactivity with serum from 10-day old micro mini pigs was observed.
No cross-reactivity was observed with cow serum.
Reactivity with the serum from other young animals (less than 3 weeks of age) has not been confirmed.

XIII. Precautions

1. Do not mix/use kits or reagents from different lots.
2. Do not expose reagents to strong light during storage or reaction.
3. Use pipettes that are free of metal when handling (5) Substrate Solution (TMBZ).
4. Prevent (5) Substrate Solution (TMBZ) from coming into contact with hands or mucous membranes.
5. Do not use (5) Substrate Solution (TMBZ) that has developed color.
6. Each reaction varies depending on time and temperature. Therefore, a new standard curve must be generated for each assay.
7. Handle blood samples with great care.

IX. Related Products

Human Albumin EIA Kit (Cat. #MK132)
Monoclonal Antibody to Human Alpha Fetoprotein (Cat. #M225)
Monoclonal Antibody to Human Albumin (Cat. #M226)
Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021)
Personal Microplate Washer (Cat. #MK950)＊

＊: Not available in all geographic locations. Check for availability in your region.