Quantikine® ELISA

Mouse α-Fetoprotein/AFP Immunoassay

Catalog Number MAFP00

For the quantitative determination of mouse alpha-Fetoprotein (AFP) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

α-Fetoprotein (AFP), a member of the albuminoid superfamily (Albumin, Vitamin D-binding protein, and α-Albumin), is a fetal- and tumor-associated protein well known as a marker for certain cancers and congenital defects (1-4). In humans and rodents, it is a single chain glycoprotein of 66-72 kDa, with 3-5% of the molecular weight resulting from glycosylation (3). Mouse AFP shares 81% and 65% amino acid sequence identity with rat and human AFP, respectively. AFP is synthesized in the fetus primarily by the liver, yolk sac, and tissues of gastrointestinal origin (5-8). During mouse development, AFP is expressed at low but detectable levels in the foregut endoderm, becomes highly activated in hepatoblasts that form the liver bud, and is one of the earliest markers of the hepatocyte lineage (9). Fetal serum levels peak at weeks 10 through 13 and then decline throughout gestation (10). In contrast, maternal serum AFP, derived primarily from fetal circulation, continues to increase into the third trimester before declining (10).

AFP can act as a carrier protein, binding several ligands including steroids, bilirubin, fatty acids, retinoids, and flavonoids (4). In rodents, AFP sequesters endogenous estrogen and blocks the induction of estrogen receptor (ER)-mediated responses (2). Ligand binding may affect the rigidity and conformation of the AFP tertiary structure (2, 4, 11, 12). In addition, AFP has been proposed to have immunosuppressive activity, regulate cell proliferation and apoptosis, initiate intracellular signaling, and contribute to cell invasion (4, 13). Putative cell surface receptors for AFP have been described, and AFP is internalized by many cell types (14). AFP knockout mice are viable, but females are infertile due to an inadequate hormonal environment that blocks normal ovulation (15, 16).

Altered levels of both fetal and maternal AFP have been associated with several congenital abnormalities including hypothyroidism, autoimmune disorders, and heart defects (2, 17-19). In addition, AFP is widely used as a prenatal screening marker for neural tube defects and chromosomal abnormality (2, 20). Low maternal serum AFP levels have been associated with a higher incidence of Down's syndrome, whereas higher levels are associated with spina bifida and anencephaly (21-24). Postnatal production of AFP is associated with several pathologies. AFP is a commonly used marker for hepatocellular cancer, and elevated AFP levels are coincident with several other cancers including hepatoblastoma, germ cell tumors, and certain gastric cancers (25-31).

The Quantikine Mouse α-Fetoprotein/AFP Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse AFP levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse AFP and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse AFP accurately. Results obtained using natural mouse AFP showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse AFP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse AFP has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse AFP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse AFP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse AFP bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse AFP Microplate	894749	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse AFP.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse AFP Standard	894751	2 vials of recombinant mouse AFP in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard and control for each assay.
Mouse AFP Control	894752	2 vials of recombinant mouse AFP in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse AFP Conjugate	894750	12 mL of a polyclonal antibody specific for mouse AFP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse AFP Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

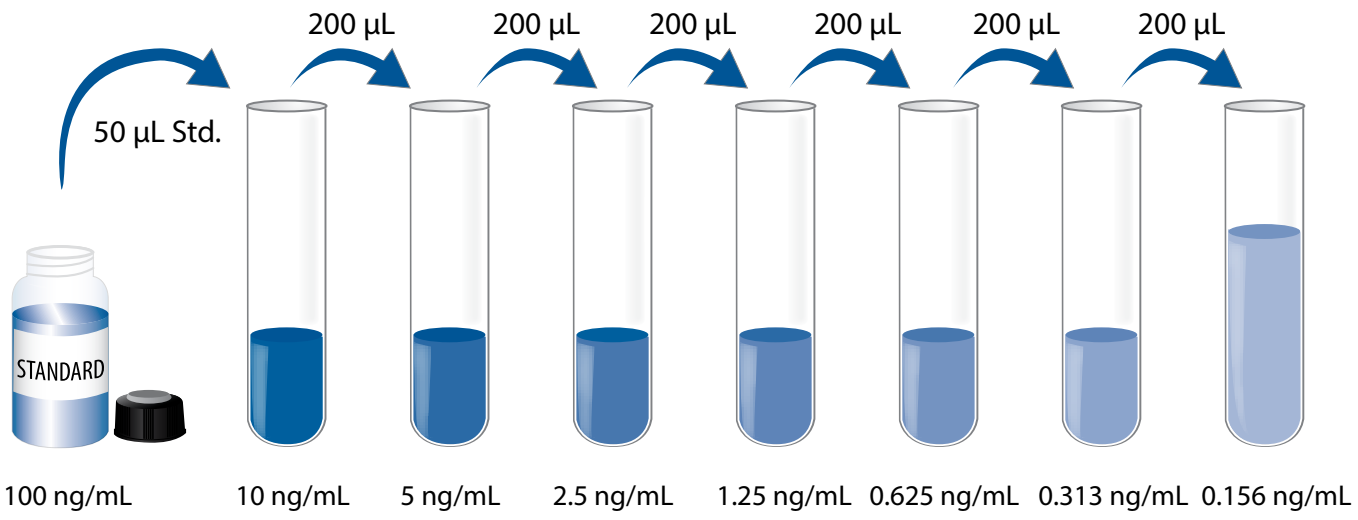
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse AFP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse AFP Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 10 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, Control, 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, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse AFP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

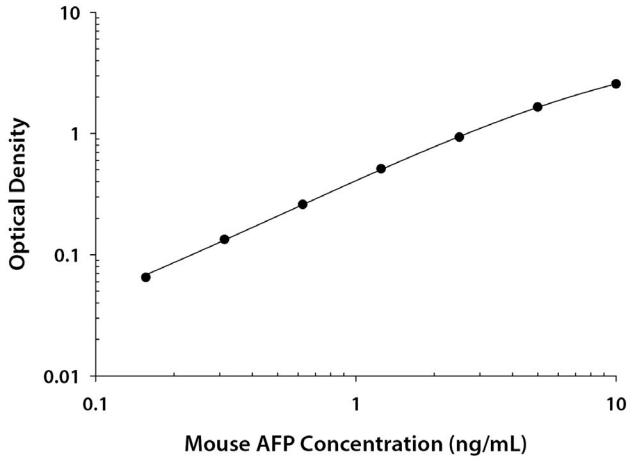
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse AFP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.014 0.016	0.015	—
0.156	0.078 0.082	0.080	0.065
0.313	0.145 0.152	0.149	0.134
0.625	0.270 0.279	0.275	0.260
1.25	0.513 0.538	0.526	0.511
2.5	0.929 0.968	0.949	0.934
5	1.635 1.694	1.665	1.650
10	2.545 2.611	2.578	2.563

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)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.404	1.12	3.30	0.457	1.20	3.38
Standard deviation	0.013	0.026	0.091	0.031	0.066	0.158
CV (%)	3.2	2.3	2.8	6.8	5.5	4.7

RECOVERY

The recovery of mouse AFP spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	93-105%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse AFP were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	103	98	99
	Range (%)	94-100	98-107	96-101	98-100
1:4	Average % of Expected	98	102	97	100
	Range (%)	93-107	95-107	94-101	99-101
1:8	Average % of Expected	99	101	98	99
	Range (%)	91-111	95-107	94-103	97-102
1:16	Average % of Expected	95	99	96	96
	Range (%)	88-109	96-102	95-100	92-98

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SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of mouse AFP ranged from 0.003-0.029 ng/mL. The mean MDD was 0.007 ng/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-derived recombinant mouse AFP produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse AFP in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	39.2	13.8-63.4	15.1
EDTA plasma (n=5)	34.0	21.5-56.2	14.1
Heparin plasma (n=5)	48.5	27.1-80.5	21.6

Cell Culture Supernates - Hepa 1-6 mouse hepatoma cells (1 x 10^6 cells/mL) were cultured for 6 days in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse AFP, and measured 426 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse AFP.

The factors listed below were prepared in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors in a mid-range recombinant mouse AFP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Albumin
α-Fetoprotein

Other factors:

human bilirubin
human hemoglobin
mouse serum albumin
human triolein

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