Quantikine® ELISA

Human α-Fetoprotein Immunoassay

Catalog Number DAFP00

For the quantitative determination of human Alpha Fetoprotein (AFP) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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/tumor associated protein well known as a marker for certain cancers and congenital defects (1-3). In humans and rodents, it is a single chain glycoprotein of 66-72 kDa, 3-5% of the molecular weight resulting from glycosylation (3). AFP is synthesized in the fetus primarily by the liver, yolk sac, and tissues of gastrointestinal origin (4-7). Fetal serum levels peak at weeks 10 through 13 and then decline throughout gestation (8). In contrast, maternal serum AFP, derived primarily from fetal circulation, continues to increase into the third trimester before declining (8).

Biological functions of AFP continue to be elucidated. Like other members of the albuminoid family, AFP can act as a carrier protein, binding several ligands including steroids, bilirubin, fatty acids, retinoids, and flavonoids (3). Ligand binding may affect the rigidity and conformation of the AFP tertiary structure (2, 9, 10). In addition, some studies suggest that AFP may act as a regulator of cell growth. AFP has growth inhibitory and apoptotic effects on several human tumor cell lines, potentially involving Caspase-dependent mechanisms (11, 12). Furthermore, peptides derived from AFP can suppress tumor growth (13, 14). Growth inhibitory effects are context-dependent since AFP can stimulate cell growth in certain cell types, both alone and in synergy with growth factors (11, 15-17). How AFP signals are transmitted intracellularly is unclear, although putative cell surface receptors have been described, and AFP is internalized by many cell types (18-22). Knockout mice are viable despite prominent fetal AFP expression (23). Although early development appears intact, $AFP^{-/-}$ females are infertile, potentially due to defects in the hypothalamic/pituitary system (23).

Altered levels of both fetal and maternal AFP have been associated with several congenital abnormalities including hypothyroidism, autoimmune disorders, and heart defects (2). In addition, AFP is a widely used marker indicative of chromosomal or neural tube abnormality (2). Low maternal serum AFP levels have been associated with a higher incidence of Down's syndrome (24-26), whereas higher levels are associated with spina bifida and anencephaly (3, 27-29). Certain pathological conditions can trigger AFP production postnatally. Elevated AFP levels are coincident with several cancers including hepatoblastoma, hepatocellular carcinoma, germ cell tumors, and certain gastric cancers (30-35). In addition, AFP can be elevated in benign hepatocellular diseases including active hepatitis and cirrhosis (36, 37).

The Quantikine® Human α-Fetoprotein Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human AFP in cell culture supernates, serum, and plasma.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human AFP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AFP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human AFP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of AFP bound in the initial step. The color development is stopped and the intensity of the color is measured.

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, dilute the samples with the appropriate 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human AFP Microplate	892821	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human AFP Conjugate	892822	21 mL of a polyclonal antibody specific for human AFP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human AFP Standard	892823	Human AFP in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* **Note:** *Human sourced material. See Precautions section.*	
Assay Diluent RD1-82	895375	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a buffer with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD5-33	895813	21 mL of a buffered protein base with preservatives. *For serum/plasma samples.*	
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	895032	6 mL of 2 N sulfuric 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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Human AFP Standard provided with this kit was prepared from human cord serum. This AFP was tested at the donor level using FDA licensed methods and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, this reagent should be handled as if capable of transmitting infection.

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

Some components in this kit contain a preservative 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. 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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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 15 minutes at 1000 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.*
Hemolyzed samples are not suitable for use in this assay.
High levels of serum albumin may interfere in this assay.

REAGENT PREPARATION

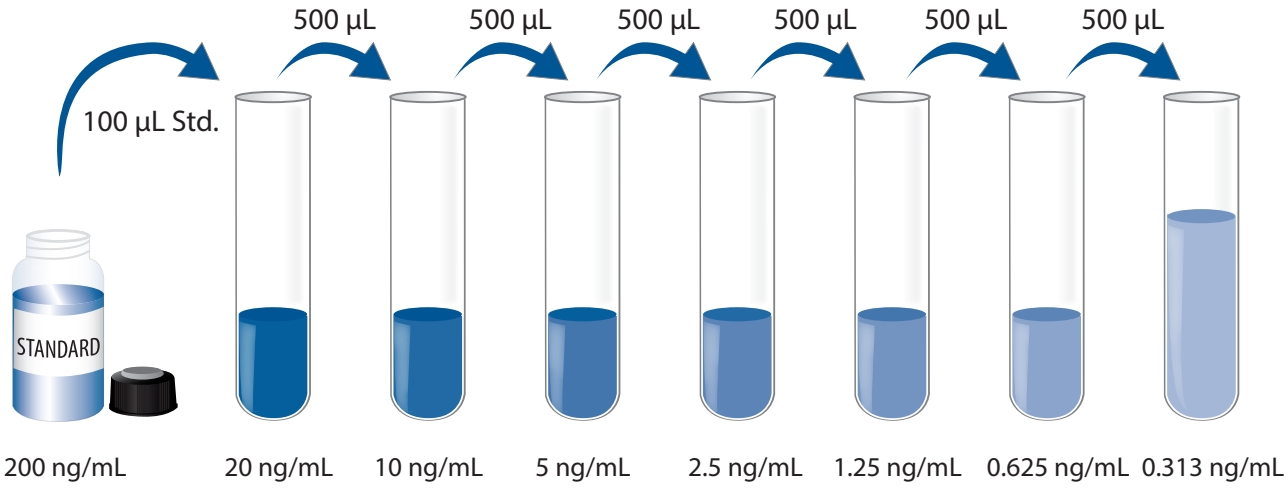
Bring all reagents to room temperature before use.

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.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human AFP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human AFP Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-24 (*for cell culture supernate samples*) or Calibrator Diluent RD5-33 (*for serum/plasma samples*) into the 20 ng/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The appropriate calibrator diluent 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 samples, controls, and standards be assayed 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, and reseal.
3. Add 100 µL of Assay Diluent RD1-82 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. A plate layout is provided to record standards and samples assayed.
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 200 µL of Human AFP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.

CALCULATION OF RESULTS

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

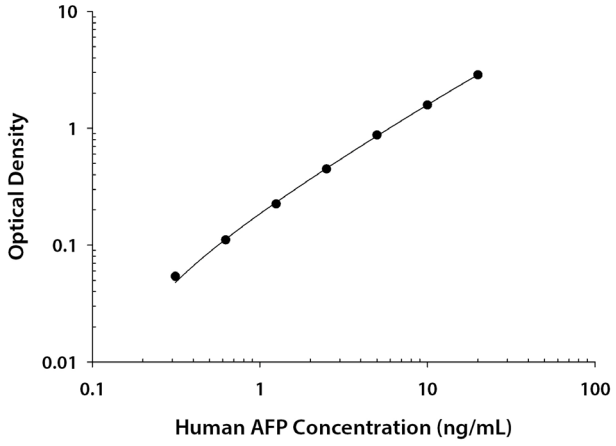
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human 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

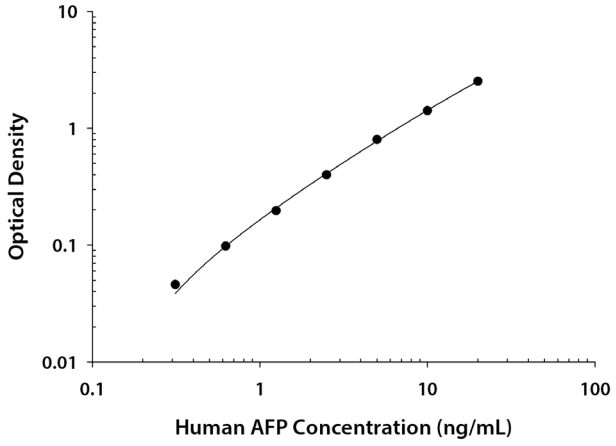
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
0.313	0.064 0.067	0.066	0.054
0.625	0.122 0.124	0.123	0.111
1.25	0.233 0.240	0.237	0.225
2.5	0.450 0.467	0.459	0.447
5	0.885 0.886	0.886	0.874
10	1.545 1.638	1.592	1.580
20	2.861 2.890	2.876	2.864

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.012 0.012	0.012	—
0.313	0.054 0.061	0.058	0.046
0.625	0.104 0.115	0.110	0.098
1.25	0.207 0.210	0.209	0.197
2.5	0.410 0.412	0.411	0.399
5	0.803 0.819	0.811	0.799
10	1.417 1.433	1.425	1.413
20	2.531 2.543	2.537	2.525

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	2.89	6.02	12.3	2.65	5.62	11.7
Standard deviation	0.109	0.219	0.441	0.158	0.261	0.549
CV (%)	3.8	3.6	3.6	6.0	4.6	4.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	3.15	6.62	13.2	3.22	6.79	14.1
Standard deviation	0.080	0.149	0.447	0.215	0.382	0.930
CV (%)	2.5	2.3	3.4	6.7	5.6	6.6

RECOVERY

The recovery of human AFP spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	95-103%
Serum (n=4)	103	86-115%
EDTA plasma (n=4)	104	94-114%
Heparin plasma (n=4)	103	95-115%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human AFP were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	108	101	97	99
	Range (%)	107-110	98-104	92-102	92-103
1:4	Average % of Expected	102	96	93	95
	Range (%)	100-106	92-102	87-100	92-102
1:8	Average % of Expected	95	94	89	91
	Range (%)	94-99	90-98	85-94	87-101
1:16	Average % of Expected	96	94	92	91
	Range (%)	91-98	89-100	88-98	87-99

SENSITIVITY

One hundred ten assays were evaluated and the minimum detectable dose (MDD) of human AFP ranged from 0.004-0.046 ng/mL. The mean MDD was 0.013 ng/mL.

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

CALIBRATION

This immunoassay is calibrated to the NIBSC/WHO International Standard for AFP (72/225), which was prepared with cord blood.

The dose response curve of the International Standard (72/225) parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human AFP kit to approximate NIBSC 72/225 units, use the equation below.

NIBSC (72/225) approximate value (IU/mL) = 0.8056 x Quantikine® Human AFP value (ng/mL).

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human AFP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum* (n=36)	2.57	0.68 - 6.9	1.5
EDTA plasma (n=36)	2.51	0.75 - 7.0	1.5
Heparin plasma (n=36)	2.46	0.80 - 7.6	1.5

*Three additional samples were run. Two samples were within the normal range, and one sample read 16.3 ng/mL.

Cell Culture Supernates:
Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of human AFP. No detectable levels were observed.

HepG2 human hepatocellular carcinoma cells were cultured for 24 hours in MEM supplemented with 5% fetal bovine serum until confluent and stimulated with 50 ng/mL PMA. An aliquot of the cell culture supernate was removed, assayed for human AFP, and measured 2210 ng/mL.

SPECIFICITY

This assay recognizes natural human AFP.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range human AFP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Chorionic Gonadotropin (HCG)
Follicle Stimulating Hormone (FSH)
Leptin
Luteinizing Hormone (β-subunit) (LH)
Prolactin
Resistin
Thyroid Stimulating Hormone (TSH)

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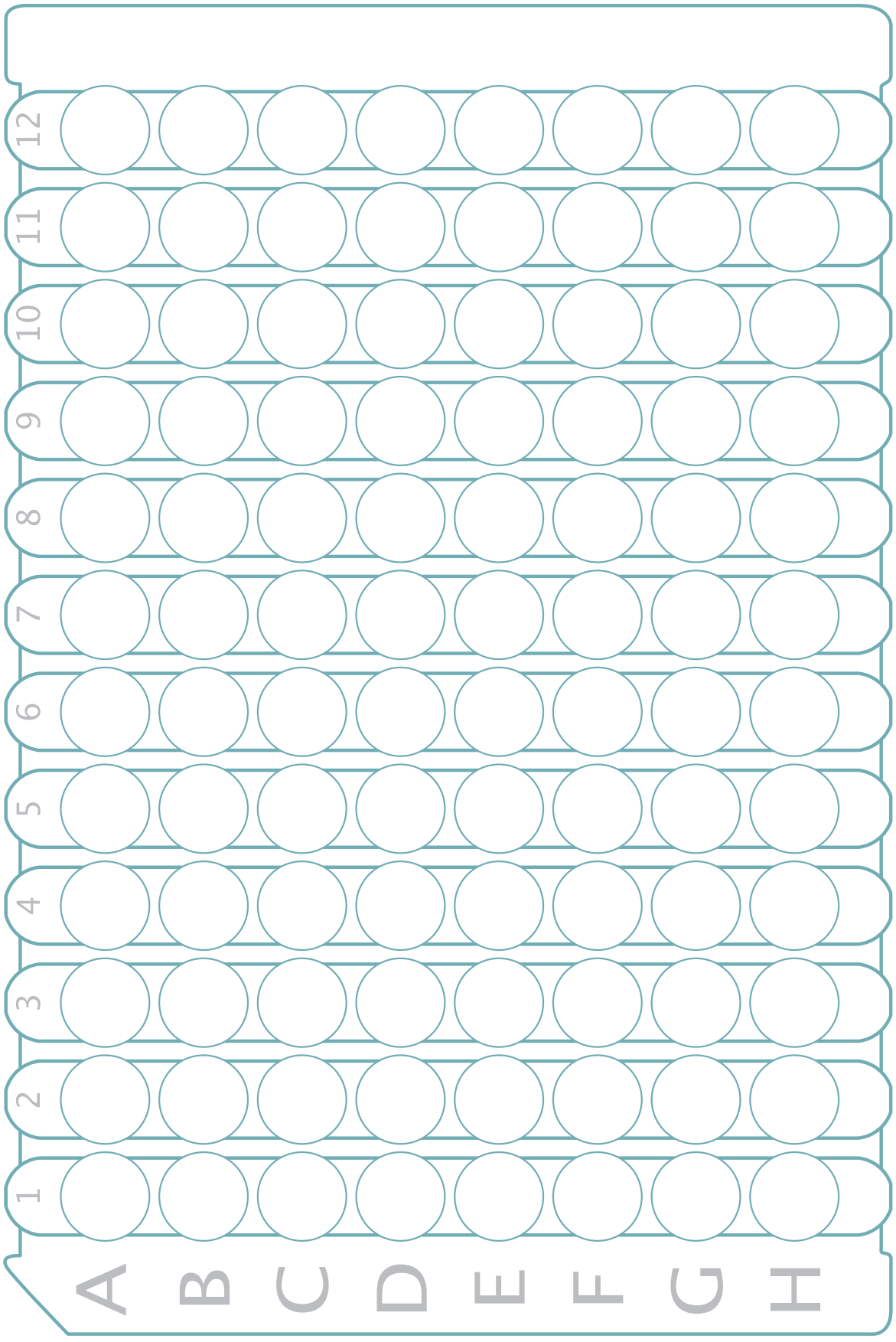
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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