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R&D SYSTEMS

Saliva Sensitive

Quantikine™ QuicKit™ ELISA

Human FAP Immunoassay

Catalog Number QK3715
SK3715
PK3715

For the quantitative determination of human FAP 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

Fibroblast activation protein (FAP), also known as seprase, is a type II transmembrane cell surface protein that promotes tumor development and metastasis by influencing extracellular matrix degradation, intracellular signaling, angiogenesis, and immunosuppression. FAP is a 170 kDa serine protease present in the form of a dimer and possesses both dipeptidyl peptidase and endopeptidase activities, enabling it to degrade gelatin and type I collagen. This proteolytic activity of FAP supports proliferation and tumor growth, making it a specific marker for tumor-associated fibroblasts and a target for novel anticancer therapies, including immune-based approaches.

Although FAP levels are low or undetectable in normal adult tissues, they are expressed at higher levels by activated fibroblasts in conditions such as cancer. Conversely, circulating FAP levels are shown to be significantly lower in cancer patients compared to healthy individuals, with increased levels being linked to improved survival. Additionally, studies show that FAP is temporarily overexpressed in normal embryonic mesenchymal tissues and during wound healing (1-6).

Given that FAP expression is highly specific to tumor fibroblasts, its detection and measurement are particularly valuable for both research and diagnostic purposes. To this end, ELISA offers a precise and quantitative method for measuring FAP activity levels across various samples.

The Quantikine™ QuicKit™ Human FAP Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human FAP levels in cell culture supernates, serum, and plasma. It contains recombinant human FAP and antibodies raised against the recombinant protein. Results obtained for naturally occurring human FAP showed linear curves that were parallel to the standard curves obtained using the recombinant QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural FAP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked polyclonal detection antibody, specific for human FAP. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of FAP bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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 #	CATALOG # QK3715	CATALOG # SK3715	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 FAP Standard	899563	2 vials	12 vials	Recombinant human FAP in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a freshly reconstituted standard for each assay. Discard after use.
Human FAP Capture Ab Concentrate	899561	1 vial	6 vials	Lyophilized tagged monoclonal antibody specific for human FAP.	May be stored for up to 1 month at 2-8 °C.* **Do not freeze after reconstitution**
Assay Diluent RD1-116	895858	1 vial	6 vials	11 mL of a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	Undiluted may be stored for up to 1 month at 2-8 °C.* Diluted, discard after use.
Human FAP Detection Ab Concentrate	899562	1 vial	6 vials	400 µL of a polyclonal antibody specific for human FAP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6-43	895548	1 vial	6 vials	21 mL of a buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
TMB ELISA Substrate	642736	1 vial	6 vials	12 mL of a TMB ELISA substrate.	
ELISA Stop Solution	642827	1 vial	6 vials	12 mL of an acid solution.	
Plate Sealers	N/A	4 strips	8 strips	Adhesive strips.	

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

QK3715 contains sufficient materials to run an ELISA on one 96 well plate.
SK3715 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, # PK3715). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak has enough reagents to assay 50 microplates (96 wells/plate). Although the package inserts are the same as those for the single kit inserts, there are minor differences related to the number of reagents and their container sizes.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase* (R&D Systems®, # WA126).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
QuicKit™ Coated Microplate	899063	50 plates
Human FAP Standard*	899563	25 vials
Human FAP Capture Ab Concentrate	899561	50 vials
Human FAP Detection Ab Concentrate	899562	50 vials
Assay Diluent RD1-116	895858	50 vials
Calibrator Diluent RD6-43	895548	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642736	50 vials
ELISA Stop Solution	642827	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- 50 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
- Human FAP Controls (optional; Catalog # QC313)

PRECAUTIONS

FAP is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS 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.*

SAMPLE PREPARATION

Cell culture supernates can be tested neat. Some supernates may contain high levels of FAP and may require dilution. Multiple dilutions are recommended.

Serum and plasma samples require a 40-fold dilution. A suggested 40-fold dilution can be achieved by adding 10 µL of sample to 390 µL of Calibrator Diluent RD6-43 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *FAP is found in saliva. Wear a face mask and gloves to protect kit reagents from contamination.*

Assay Diluent RD1-116 (diluted 1:2) - Dilute 5 mL of Assay Diluent RD1-116 into 5 mL of deionized or distilled water to prepare 10 mL of Assay Diluent RD1-116 (diluted 1:2).
Note: *Make fresh prior to assay.*

Calibrator Diluent RD6-43 (diluted 1:4) - Dilute 5 mL of Calibrator Diluent RD6-43 into 15 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-43 (diluted 1:4).

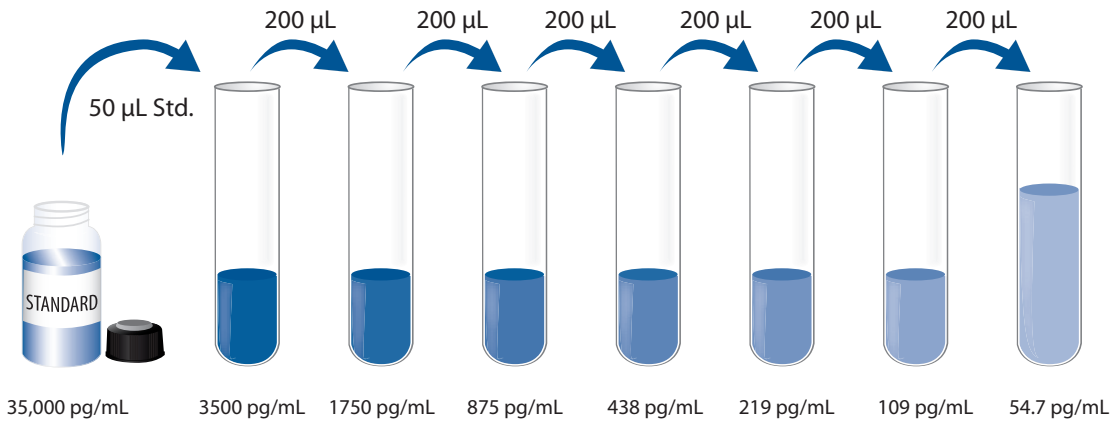
Human FAP Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FAP Capture Ab Concentrate with Assay Diluent RD1-116 (diluted 1:2). This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-116 (diluted 1:2). For a full plate, add 300 µL of reconstituted Human FAP Capture Ab stock and 300 µL of Human FAP Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-116 (diluted 1:2).

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Human FAP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FAP Standard with distilled or deionized water. This reconstitution produces a stock solution of 35,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD6-43 (diluted 1:4) into the 3500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 3500 pg/mL standard serves as the high standard. Calibrator Diluent RD6-43 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

Note: *FAP is found in saliva. Wear a face mask and gloves to protect kit reagents from contamination.*

1. Prepare all reagents, samples, and working standards 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 standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour 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 twice for a total of three 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 TMB ELISA Substrate solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 100 µL of ELISA 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.
8. 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

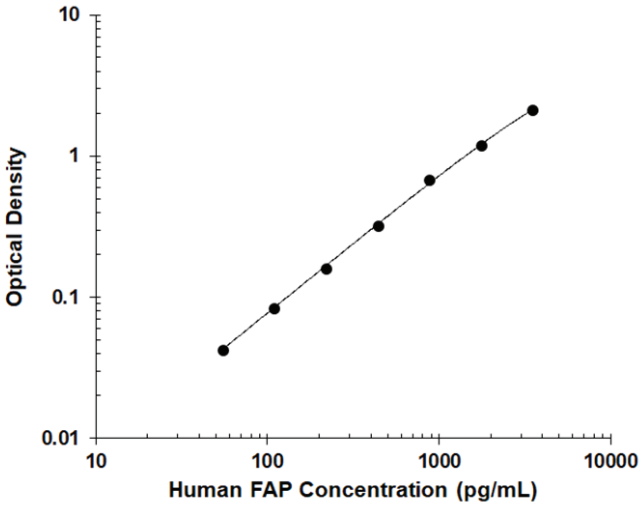
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human FAP 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.



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.026	0.024	—
54.7	0.066 0.066	0.066	0.042
109	0.106 0.108	0.107	0.083
219	0.184 0.184	0.184	0.160
438	0.341 0.348	0.345	0.321
875	0.658 0.741	0.700	0.676
1750	1.206 1.216	1.211	1.187
3500	2.145 2.170	2.158	2.134

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least five technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	283	1511	315	1835
Standard deviation	14.1	103	34.4	84.2
CV (%)	5.0	6.8	10.9	4.6

RECOVERY

The recovery of human FAP spiked to three different levels in samples throughout the range of the assay in cell culture media was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	94-108%

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of human FAP ranged from 3.56-17.6 pg/mL. The mean MDD was 9.01 pg/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.

LINEARITY

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

		Cell culture media (n=4)	Serum* (n= 2)	EDTA plasma* (n= 2)	Heparin plasma* (n= 2)
1:2	Average % of Expected	98	96	93	97
	Range (%)	95-100	93-99	90-96	95-98
1:4	Average % of Expected	95	97	97	91
	Range (%)	92-98	92-102	94-100	84-98
1:8	Average % of Expected	94	97	94	104
	Range (%)	91-97	92-102	94-95	97-111
1:16	Average % of Expected	90	102	100	109
	Range (%)	84-96	99-105	94-107	107-111

* Samples were diluted prior to assay.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human FAP produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	59,707	37,213-88,897	16,910
EDTA plasma (n=10)	56,723	40,866-79,171	12,744
Heparin plasma (n=10)	60,481	35,740-92,744	16,049

Cell Supernates: WI-38 cells were cultured in MEM NEAA Earle's Salts supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 ug/mL streptomycin sulfate, and 1 mM sodium pyruvate until nearly confluent. Cells were then left untreated or treated with 10 ng/mL rhTGF-β, 1 mM Retinoic acid, or 60 nM PMA prior to harvest. Aliquots of the cell culture supernates were removed and assayed for human FAP.

Condition	Concentration (pg/mL)
Unstimulated WI-38 cells	253
WI-38 with TGF-β	1018
WI-38 with Retinioic acid	289
WI-38 with PMA	449

SPECIFICITY

This assay recognizes natural and recombinant human FAP.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level recombinant human FAP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ACE
ACE-2
CD26
DPP-10
DPP-6
DPP-9
ECE-1
ECE-2
Neprilysin
THY-1
Urokinase R

Recombinant mouse:

CD26
FAP

Recombinant cynomolgus macaque:

CD26

Recombinant cynomolgus macaque FAP cross-reacts approximately 46% and interferes at concentrations greater than 625 pg/mL in this assay.

REFERENCES

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3. Christiansen, VJ. *et al*. (2013) Neoplasia. **15**:348.
4. Cheng, JD. *et al.* (2005) Mol Cancer Ther. **4**:351.
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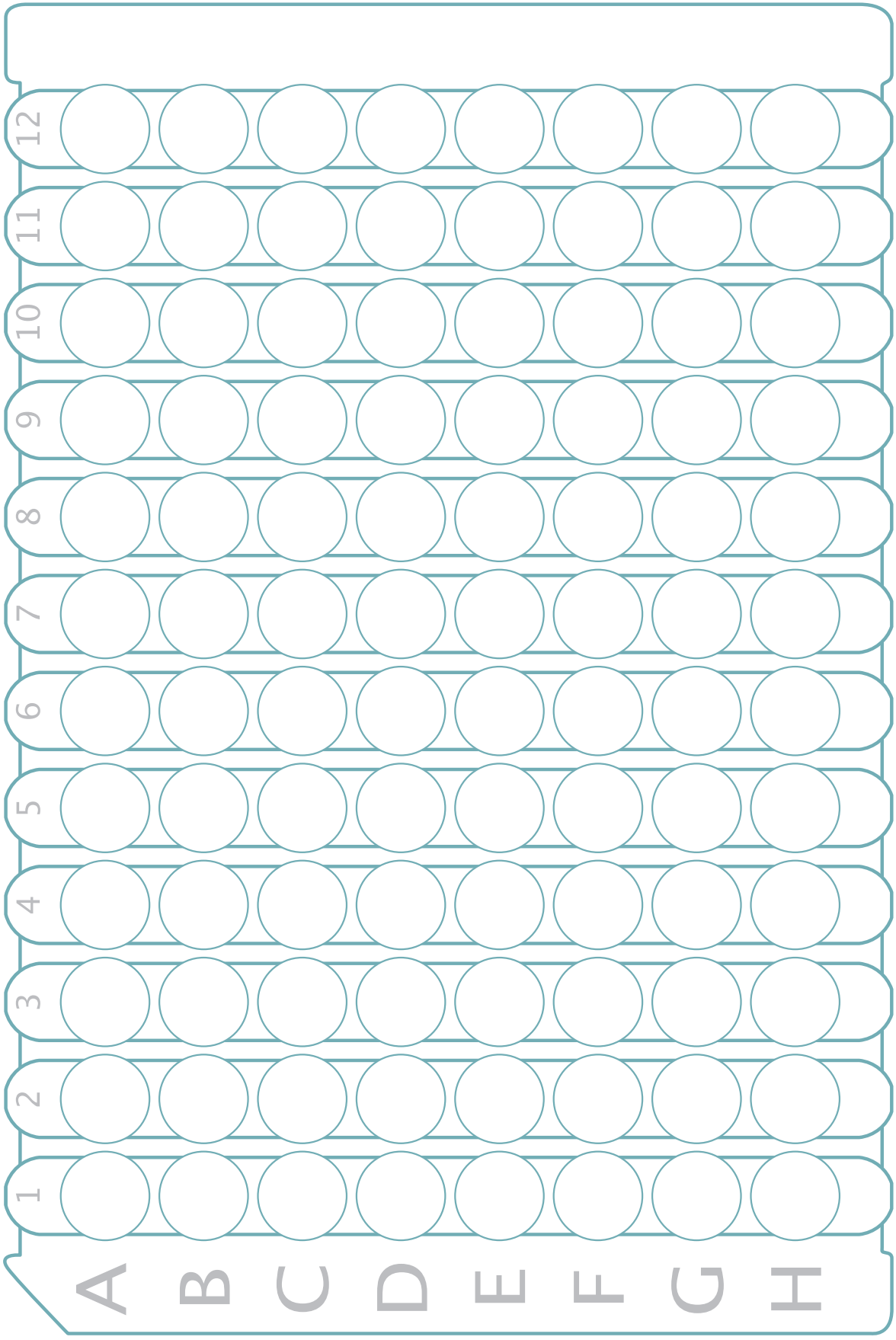
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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