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R&D SYSTEMS™

Quantikine™ ELISA

Human sTfR Immunoassay

Catalog Number DTFRU0
STFRU0
PDTFRU0

For the quantitative determination of soluble human transferrin receptor (sTfR) concentrations in 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

Transferrin Receptor (TfR) is the major mediator of iron uptake by cells (1, 2). TfR is a transmembrane, disulfide-linked dimer of two identical subunits (3-7) that binds and internalizes diferric transferrin, thereby delivering iron to the cell cytosol. When a cell needs iron, TfR expression is increased to facilitate iron uptake (8-10). Since the major use of iron is for hemoglobin synthesis, about 80% of total TfR is on erythroid progenitor cells (1, 2).

Soluble Transferrin Receptor (sTfR) arises from proteolysis of TfR at a specific site in the extracellular domain, leading to monomers that can be measured in plasma and serum (11, 12). A constant relationship has been reported between total TfR and the concentration of sTfR in plasma or serum (13). Thus, the concentration of sTfR in plasma or serum is an indirect measure of total TfR.

Since TfR expression is increased in iron deficiency and since most TfR is on erythroid progenitor cells, the serum level of sTfR reflects either the cellular (primarily erythroid) need for iron (14-19), or the size of the erythroid progenitor pool (*i.e.*, the rate of erythropoiesis) (2, 14, 20). Two lines of evidence support this. First, the concentration of sTfR in plasma or serum is elevated in iron deficiency (14-19, 21). Second, the concentration of sTfR in plasma or serum is correlated with the Erythron Transferrin Uptake (2), a ferrokinetic measure of erythropoietic activity, and sTfR is elevated in subjects with hyperplastic erythropoiesis (*e.g.*, hemolytic anemia, thalassemia, polycythemia, etc.) and depressed in subjects with hypoplastic erythropoiesis (e.g., chronic renal failure, aplastic anemia or post-transplant anemia) (14, 20).

The Quantikine™ Human sTfR Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human sTfR in serum and plasma. Results obtained using natural human sTfR showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human sTfR.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human sTfR has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sTfR present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human sTfR 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 sTfR 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 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™ 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 #	CATALOG # DTFRU0	CATALOG # STFRU0	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human sTfR Microplate	899549	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human sTfR.	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 sTfR Standard	899551	2 vials	6 vials	Human sTfR in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human sTfR Conjugate	899550	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human sTfR conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-135	897671	2 vials	12 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6-70	897672	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DTFRU0 contains sufficient materials to run an ELISA on one 96 well plate.
STFRU0 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, # PDTFRU0). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts are the same as those in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- 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
Human sTfR Microplate	899549	50 plates
Human sTfR Standard*	899551	25 vials
Human sTfR Conjugate	899550	50 vials
Assay Diluent RD1-135	897671	75 vials
Calibrator Diluent RD6-70	897672	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	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
- 50 mL and 500 mL graduated cylinders
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- Test tubes for dilution of standards
- Human sTfR Controls (optional; R&D Systems®, # QC302)

PRECAUTIONS

The sTfR Standards and Controls contain human sTfR. This sTfR was tested at the donor level using an FDA licensed method 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, these reagents 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 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.

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 this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Samples require a 2-fold dilution in Calibrator Diluent RD6-70 prior to assay. An example dilution is 100 µL of sample and 100 µL of Calibrator Diluent RD6-70. Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

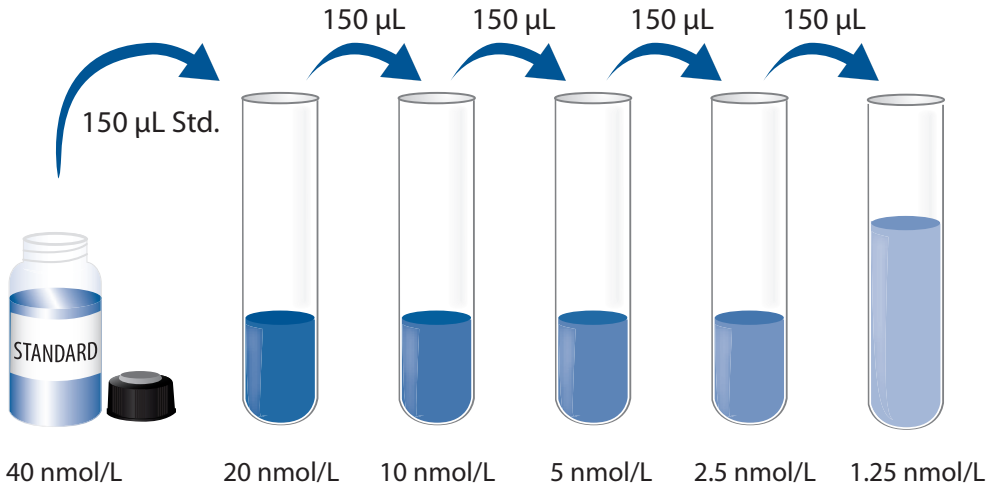
Bring all reagents to room temperature before use.

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 480 mL of 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 sTfR Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human sTfR Standard with Calibrator Diluent RD6-70. This reconstitution produces a stock solution of 40 nmol/L. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 150 µL of Calibrator Diluent RD6-70 into five tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 40 nmol/L reconstituted standard serves as the high standard. Calibrator Diluent RD6-70 serves as the zero standard (0 nmol/L).



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.

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 150 µL of Assay Diluent RD1-135 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. A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 sTfR Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 on the benchtop. 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.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

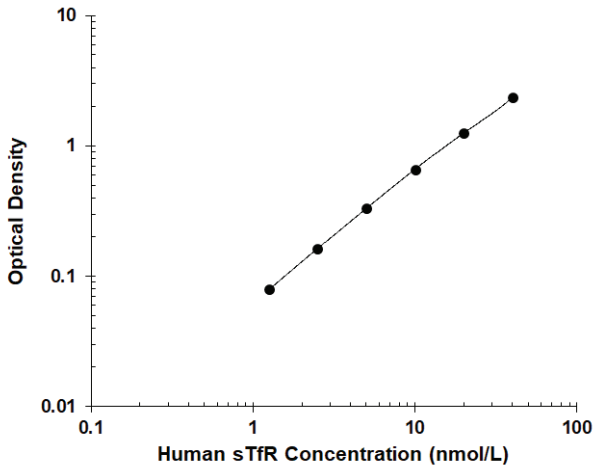
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 sTfR 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.

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.



(nmol/L)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
1.25	0.087 0.088	0.088	0.079
2.5	0.168 0.173	0.171	0.162
5	0.339 0.342	0.341	0.332
10	0.659 0.681	0.670	0.661
20	1.252 1.278	1.265	1.256
40	2.343 2.403	2.373	2.364

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 (nmol/L)	4.82	18.4	28.8	5.02	19.8	29.5
Standard deviation	0.115	0.375	0.791	0.202	1.31	2.56
CV (%)	2.4	2.0	2.7	4.0	6.6	8.7

RECOVERY

The recovery of human sTfR spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=4)	109	94-116
EDTA plasma (n=4)	107	98-114
Heparin plasma (n=4)	107	96-119

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human sTfR ranged from 0.003-0.201 nmol/L. The mean MDD was 0.064 nmol/L.

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 spiked with high concentrations of human sTfR in various matrices were diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	99	97	97
	Range (%)	98-101	94-100	95-100
1:4	Average % of Expected	96	93	94
	Range (%)	93-99	89-96	91-97
1:8	Average % of Expected	97	93	97
	Range (%)	92-104	87-100	93-100
1:16	Average % of Expected	97	95	101
	Range (%)	91-110	88-105	96-105

CALIBRATION

This immunoassay is calibrated to the WHO Reference Reagent for sTfR (07/202).

The NIBSC Human sTfR (Transferrin Receptor) Reference Reagent (07/202) was evaluated in this kit. The dose response curve of the Reference Reagent (07/202) parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human sTfR kit to approximate NIBSC 07/202 units, use the equation below.

NIBSC Human sTfR (Transferrin Receptor) Reference Reagent 07/202 approximate value (nmol/L) = 0.7331 x Quantikine Human TfR RUO value (nmol/L)

Based on data generated from May 2024.

SAMPLE VALUES

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

Sample Type	Mean (nmol/L)	Range (nmol/L)	Standard Deviation
Serum (n=30)	22.7	12.8-52.2	8.07
EDTA plasma (n=30)	21.9	12.3-50.1	8.04
Heparin plasma (n=30)	22.2	13.2-49.7	7.92

SPECIFICITY

This assay recognizes natural human sTfR.

Preparations of the following substances were tested in multiple serum sample pools for interference. The sTfR ELISA exhibits no interference with up to 30 mg/mL Triolein, 20 mg/mL Human Serum Albumin, 1 mg/mL Bilirubin, or 1.25 mg/mL Hemoglobin. Levels of Hemoglobin above 1.25 mg/mL showed some interference.

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 mid-range human sTfR control were assayed for interference. No significant cross-reactivity or interference was observed.

Human:

Holo-Transferrin
Apo-Transferrin
Ferritin

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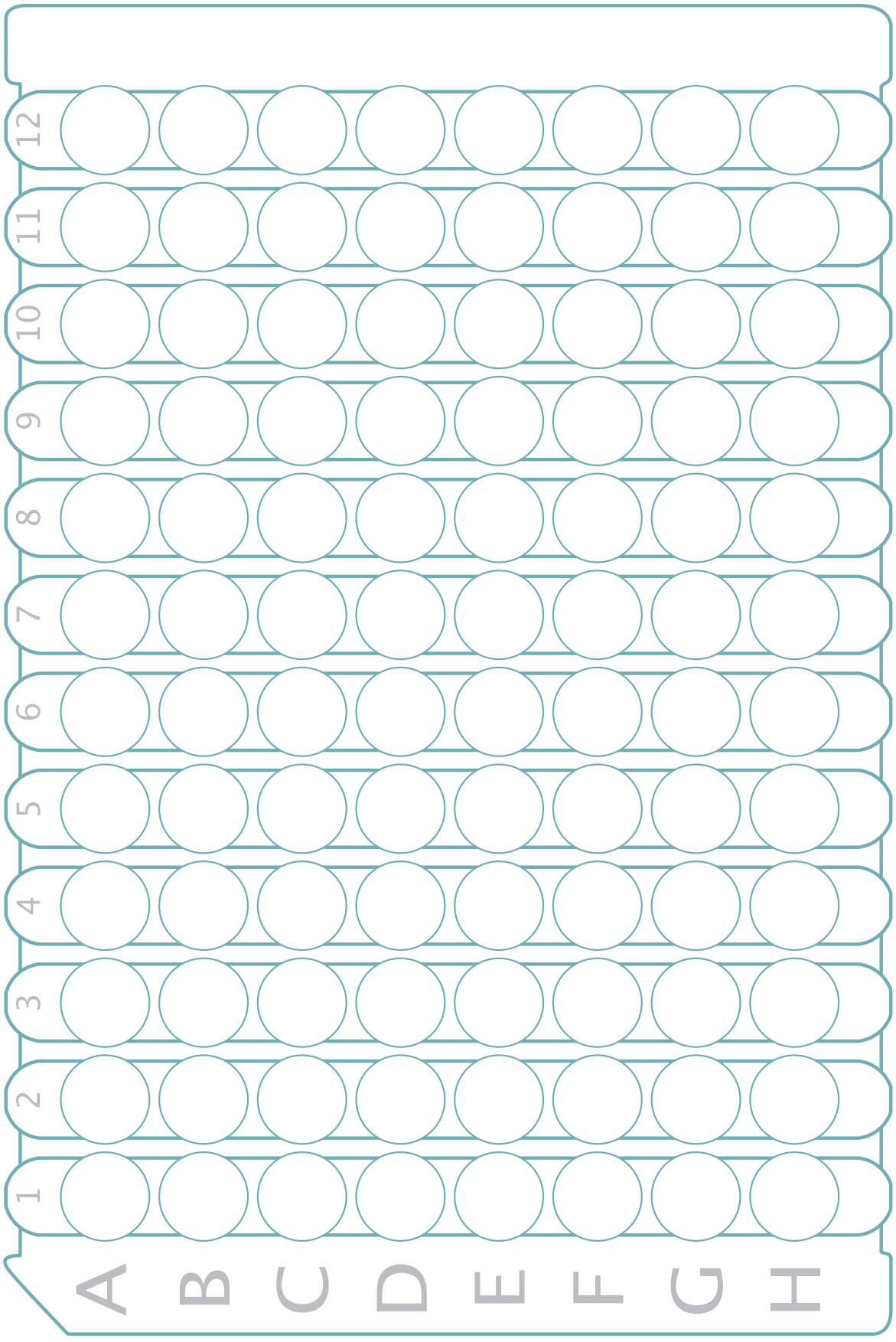
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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