Quantikine® IVD®

Human sTfR Immunoassay

REF DTFR1

This package insert must be read in its entirety before using this product.

Instructions for use in Danish, German, Spanish, French, Greek, Italian, Portuguese, and Swedish are available in the supplementary package insert.

DK Denne indlægsseddel indeholder instruktionsvejledning på engelsk. Instruktionsvejledning på dansk findes i den suplerende indlægsseddel.

DE Diese Produktbeschreibung enthält Gebrauchsvorschriften in englischer Sprache. Ein deutsches Protokoll finden Sie in der Zusatz-Packungsbeilage.

ES Este manual incluye las instrucciones de utilización en inglés. Para consultar el protocolo en español, deberán remitirse al manual suplementario.

FR La notice d'utilisation de ce produit est en anglais. Pour obtenir le protocol en français, merci de consulter la notice supplémentaire incluse avec ce produit.

GR Το παρόν ένθετο περιέχει οδηγίες χρήσης στην αγγλική γλώσσα. Για να διαβάσετε το πρωτόκολλο στην ελληνική γλώσσα, ανατρέξτε στο συμπληρωματικό ένθετο της συσκευασίας.

IT Questo inserto contiene istruzioni per l'uso in Inglese. Per il protocollo in Italiano fate riferimento all'inserto supplementare.

PT O folheto informativo deste produto contém instruções de utilização em Inglês. Para o protocolo em Português consulte por favor o folheto informativo suplementar.

SE Denna kitbroschyr är på engelska, ett kompletterande protokoll på svenska är bifogat.

IVD **FOR *IN VITRO* DIAGNOSTIC USE**

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NAME AND INTENDED USE

Quantikine® IVD® Soluble Transferrin Receptor ELISA
R&D Systems Inc. Catalog Number DTFR1

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of soluble transferrin receptor (sTfR) concentrations in human serum and plasma as an aid in the diagnosis of Iron-Deficiency Anemia (IDA), especially for the differential diagnosis of IDA and Anemia of Chronic Disease (ACD).

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, β-thalassemia, polycythemia, *etc.*) and depressed in subjects with hypoplastic erythropoiesis (*e.g.,* chronic renal failure, aplastic anemia or post-transplant anemia) (14, 20).

Measurement of sTfR is especially valuable as an indication of iron deficiency in individuals with chronic disease (inflammatory diseases, infections, malignancies), many of whom are anemic. ACD and IDA, the most common forms of anemia, are differentiated primarily by estimates of iron status (19, 22, 23). Standard measures of iron status, such as ferritin, total iron-binding capacity, and serum iron, are, however, directly affected by chronic disease, leading to equivocal results (23, 24). In contrast, sTfR is elevated in iron deficiency but is not appreciably affected by chronic disease. Thus, sTfR measurement substantially improves the laboratory diagnosis of iron deficiency, even in patients with concurrent chronic disease.

sTfR is intended for use with other parameters of iron status as an aid in diagnosis of iron deficiency. Interpretation should be made with the understanding that sTfR is also elevated by hyperplastic erythropoiesis and ineffective erythropoiesis.

PRINCIPLE OF THE ASSAY

This assay is based on the microplate sandwich enzyme immunoassay technique using two different monoclonal antibodies specific for sTfR. Samples or standards are pipetted into wells of a microplate pre-coated with a monoclonal antibody that can capture sTfR, thereby immobilizing sTfR to the well. After washing away any unbound protein, a second anti-sTfR monoclonal antibody conjugated to horseradish peroxidase is added. The conjugated antibody completes the sandwich. After washing away unbound conjugated antibody, the amount of conjugate remaining in the well is proportional to the amount of sTfR initially captured. The amount of conjugated enzyme in the well is measured by incubation with a chromogenic substrate.

LIMITATIONS OF THE PROCEDURE

- FOR *IN VITRO* DIAGNOSTIC USE.
- No drugs have been investigated for assay interference.
- The kit should not be used beyond the expiration date on the kit label.
- If samples generate values higher than the highest standard, dilute the samples with the Specimen Diluent and repeat the assay or report the values as > 80 nmol/L.
- Any variation in Specimen Diluent, operator, pipetting technique, washing technique, incubation time or temperature, or kit age can cause variation in binding.

MATERIALS PROVIDED

SORB — **sTfR Microplate** (Part 890429) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against sTfR.

CONJ — **sTfR Conjugate** (Part 890430) - 11.5 mL of mouse monoclonal antibody against sTfR conjugated to horseradish peroxidase, containing red dye and preservative.

CAL — **sTfR Standard Set** (Part 890312-890317) - 6 vials of human sTfR in 0.2 mL buffered animal serum with preservative. The concentration of sTfR is shown on the label.

CON — **sTfR Control Set** (Part 895426-895428) - 3 vials of lyophilized human sTfR in buffered animal serum with preservative.

DIL SPE — **sTfR Specimen Diluent** (Part 895429) - 5 mL of buffered animal serum with preservative.

DIL AS — **sTfR Assay Diluent** (Part 895430) - 11 mL of a buffered protein base, containing blue dye and preservative.

BUF WASH 25X — **Wash Buffer Concentrate** (Part 895199) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

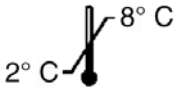
SUBS — **Substrate** (Part 895431) - 12 mL of stabilized substrate solution.

SOLN STOP — **Stop Solution** (Part 895432) - 11 mL of 1 N HCl.

Plate Covers - 4 adhesive plate sealers.

Data Card - Provides ranges of controls.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td><td rowspan="9"> 
</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="7">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>sTfR Specimen Diluent</td></tr>
<tr><td>sTfR Assay Diluent</td></tr>
<tr><td>sTfR Conjugate</td></tr>
<tr><td>Substrate</td></tr>
<tr><td>Standards</td></tr>
<tr><td>Controls</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*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 for preparation of Wash Buffer.
- Vortex mixer.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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.

SAMPLE COLLECTION AND STORAGE

The clinical utility of this test was established with serum samples, but independent measurements of serum, EDTA plasma, heparin plasma and citrate plasma* from 33 subjects gave values that were statistically indistinguishable (pairwise analysis of variance). Samples should be prepared according to CLSI/NCCLS Guideline, Procedures for the Handling and Processing of Blood Specimens (CLSI/NCCLS Document H18). Samples are stable for at least one week at 2 - 8° C, for 1 month at ≤ -20° C, or for at least 1 year at ≤ -70° C. Avoid repeated freeze-thaw cycles.

Note: *Hemolyzed samples are not suitable for measurement of sTfR with this assay.*

*Citrate plasma values must be multiplied by a dilution factor to correct for dilution by the anticoagulant.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

sTfR Controls - Reconstitute each vial with 200 µL of deionized or distilled water. Vortex. Allow Controls to sit for a minimum of 30 minutes before use. Vortex again immediately before use.

All other reagents are ready for use.

ASSAY PROCEDURE

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

1. Prepare all reagents 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 sTfR Assay Diluent to each well.

4. Add 20 µL of Standard, sample, or Control per well. **Ensure sample addition is uninterrupted and completed within 15 minutes.** Gently tap the plate frame to mix the well contents. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature (18 - 25° C).

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 by decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 µL of sTfR Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in step 5.

8. Add 100 µL of Substrate to each well. Incubate for 30 minutes at room temperature. **Avoid placing the plate in direct light.**

9. Add 100 µL of Stop Solution to each well. If 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.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents as instructed.

2. Add 100 µL sTfR Assay Diluent to each well.

3. Add 20 µL Standard, sample, or Control to each well. **Ensure sample addition is uninterrupted and completed within 15 minutes.** Incubate 1 hour at RT (18 - 25° C).

4. Aspirate and wash 4 times.

5. Add 100 µL Conjugate to each well. Incubate 1 hour at RT.

6. Aspirate and wash 4 times.

7. Add 100 µL Substrate to each well. Incubate 30 minutes at RT.

8. Add 100 µL Stop Solution to each well. Read at 450 nm within 30 minutes.
λ correction 540 or 570 nm

CALCULATION OF RESULTS

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

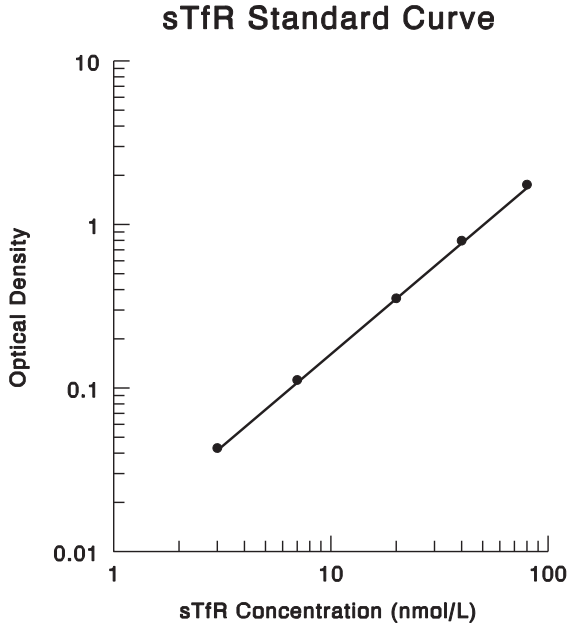
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper, and regression analysis may be applied to the log transformation.

To determine the sTfR concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

For values above the highest standard (80 nmol/L), the sample can be reported as > 80 nmol/L or it can be diluted with Specimen Diluent and re-analyzed. Values below the lowest standard (3 nmol/L) should be reported as < 3 nmol/L. Values should not be extrapolated outside the range of the standard curve.

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.	Mean	Corrected
0	0.012		
	0.013	0.012	——
3	0.055		
	0.056	0.056	0.044
7	0.123		
	0.125	0.124	0.112
20	0.365		
	0.367	0.366	0.354
40	0.825		
	0.793	0.809	0.797
80	1.756		
	1.777	1.766	1.754

QUALITY CONTROL

Each testing laboratory should establish a quality control program to monitor the performance of the Quantikine IVD sTfR Immunoassay. As part of this quality control program, controls of known sTfR concentration should be run in each assay (supplied in the kit or purchased commercially). Three controls are supplied in the kit. If the values obtained are not within their established ranges shown on the data card supplied with the kit, the assay results may be invalid.

TROUBLESHOOTING GUIDE

On the occasion of an assay failure, check the expiration dates of the individual reagents and ensure that all the reagents have been stored as indicated in the product labeling. If assay performance is questionable or a problem occurs when running the assay, you may be able to isolate the problem by referring to the following table. For further technical information call 1-800-343-7475.

Problem	Possible Source	Test or Action
Poor Precision	Incomplete washing of wells.	Ensure that the wash station is working properly.
	Inadequate aspiration of the wells.	Wells should appear dry after aspiration.
	Unequal volumes added to wells.	Ensure that the pipettes are calibrated and working properly.
	Reagent splashing during addition.	Pipette slowly and carefully.
	Inadequate mixing of reagents.	Tap the side of the plate gently to mix the well contents.
Poor Curve Fit	Pipetting error.	Consider editing data according to individual laboratory procedures.
Inadequate Color Development	Inadequate aspiration of the wells.	Wells should appear dry after aspiration.
	Incorrect volumes added to the wells.	Ensure that the pipettes are calibrated and working properly.
	Incorrect incubation times or temperatures.	Adhere to recommended incubation periods and temperatures.
	Conjugate or color reagent failure.	Mix equal volumes (*i.e.* 100 µL each) Substrate Solution and sTfR Conjugate. Color should develop immediately.

TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, 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.
- In order to minimize within assay variation, it is recommended that the Standards, samples and Controls be pipetted within 15 minutes.
- Add Standards, samples and Controls with sufficient force to ensure thorough mixing.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Additions at each step of the protocol should be uninterrupted.
- Avoid contact of Substrate with oxidizing agents or metal.
- Substrate is sensitive to light. Minimize exposure to light and keep plate out of direct light during substrate incubation.
- Substrate should remain colorless until added to the plate. Substrate incubated in the positive wells should change from colorless to gradations of blue. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Stop Solution should be added to the plate in the same order as the Substrate.

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 sixty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	4	5	6
n	20	20	20	60	60	60
Mean (nmol/L)	6.9	18.2	40.2	10.9	25.7	67.2
Standard deviation	0.3	1.3	2.5	0.7	1.4	3.8
CV (%)	4.3	7.1	6.2	6.4	5.4	5.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum	97	95 - 100%
EDTA plasma	103	97 - 112%
Heparin plasma	106	101 - 112%
Citrate plasma	112	105 - 121%

LINEARITY

To assess the linearity of the assay, five samples containing high concentrations of sTfR were diluted with Specimen Diluent and evaluated.

Dilution	% of Expected	Serum	EDTA plasma	Heparin plasma	Citrate plasma
1:2	Mean	101	98	96	97
	Range	95 - 111	93 - 103	89 - 102	90 - 105
1:4	Mean	101	100	98	92
	Range	96 - 107	90 - 109	84 - 104	84 - 96
1:8	Mean	102	99	98	95
	Range	94 - 110	88 - 105	86 - 105	90 - 108
1:16	Mean	106	106	104	98
	Range	98 - 112	102 - 111	99 - 115	89 - 116

SENSITIVITY

The minimum detectable dose (MDD) of sTfR is typically less than 0.5 nmol/L.

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

The assay is calibrated with the natural form of sTfR from human plasma. Calibration was established with highly purified plasma sTfR, with purity established by SDS-PAGE and N-terminal sequencing. The mass was obtained from amino acid analysis.

EXPECTED VALUES

Two-hundred twenty-five healthy, hematologically normal subjects from multiple locations in the United States were analyzed. sTfR values were normally distributed. Values among Blacks were statistically higher than those among non-Blacks, and subjects at high altitude (Denver, elevation ~1600 m) had higher values than those nearer sea level. There were no other statistically significant differences between races, ages, sexes, or pre- vs. post-menopausal women. **Each testing laboratory must establish its own normal range.**

	Mean (nmol/L)	2.5 - 97.5 Percentile
non-Blacks (residing < 300 m above sea level)	18.4	8.7 - 28.1*

**Blacks and those residing at 1600 m above sea level had a 6% higher 97.5 percentile value. These differences were additive.*

Serum sTfR values are above the normal range in individuals with iron-deficiency anemia and in patients with hyperplastic erythropoiesis or ineffective erythropoiesis. The sTfR assay is not intended to be used in isolation; results should be interpreted in conjunction with other diagnostic tests.

CROSS-REACTIVITY AND INTERFERENCE

Cross-reactivity

The sTfR ELISA exhibits no cross-reactivity with 8.5 mg/mL of human Diferrictransferrin, 8.5 mg/mL of human Apotransferrin, or with 10 μg/mL of human heart, liver, or spleen Ferritin.

Interference

The sTfR ELISA exhibits no interference with up to 100 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.

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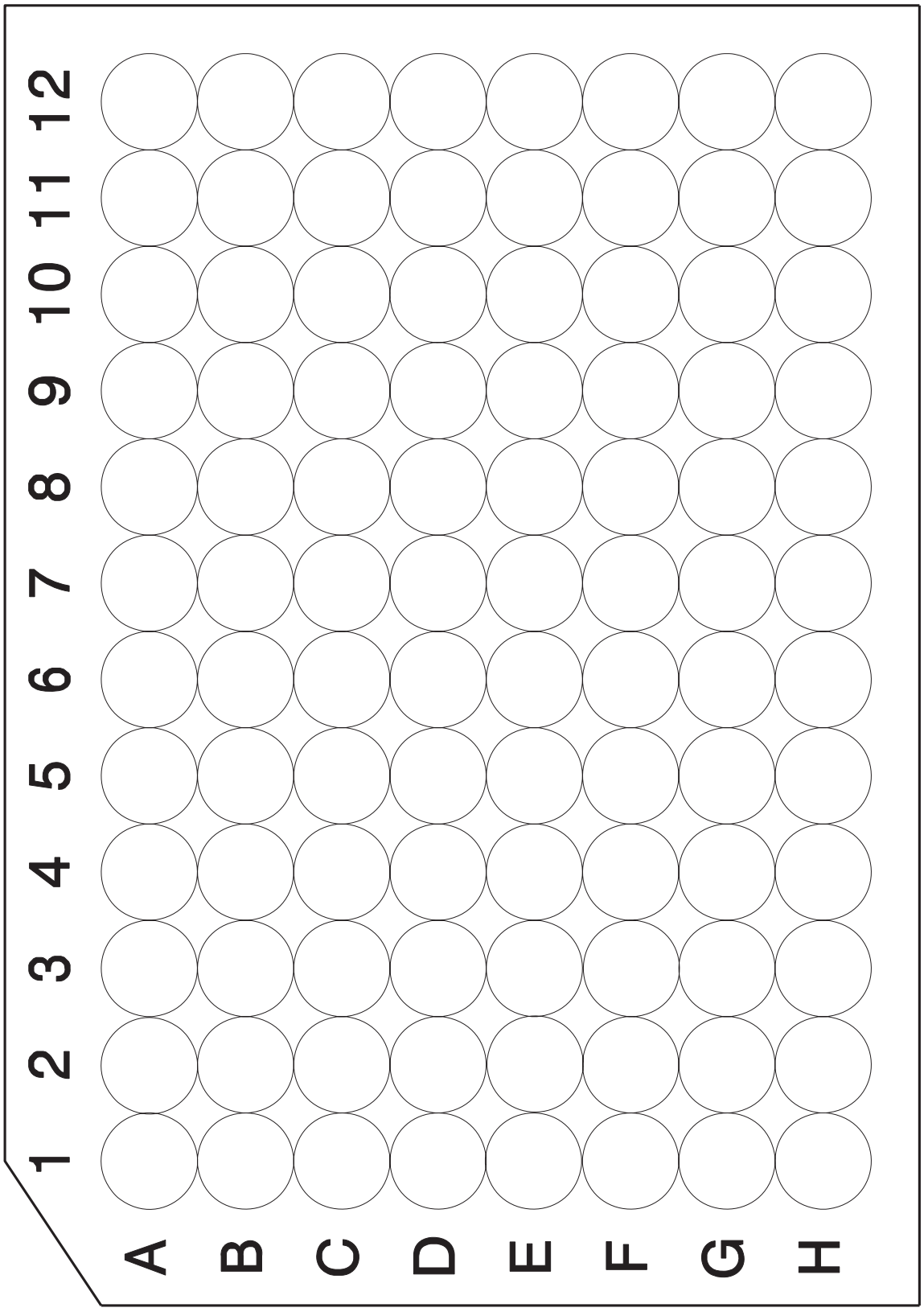
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

IVD CE