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R&D SYSTEMS

Quantikine™ ELISA

Human Erythropoietin Immunoassay

Catalog Number DEPRU0B
SEPRU0B
PDEPRU0B

For the quantitative determination of Erythropoietin (Epo) concentrations in human serum and EDTA 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

Erythropoietin (Epo), a glycoprotein (~30,400 Daltons) produced primarily by the kidney, is the principal factor regulating red blood cell production (erythropoiesis) in mammals. Renal production of Epo is regulated by changes in oxygen availability. Under conditions of hypoxia, the level of Epo in the circulation increases leading to increased production of red blood cells.

The over-expression of Epo may be associated with certain pathophysiological conditions (1, 2). Polycythemia exists when there is an overproduction of red blood cells (RBCs). Primary polycythemias, such as polycythemia vera, are caused by Epo-independent growth of erythrocytic progenitors from abnormal stem cells and low to normal levels of Epo are found in the serum of affected patients. On the other hand, various types of secondary polycythemias are associated with the production of higher than normal levels of Epo. The overproduction of Epo may be an adaptive response associated with conditions that produce tissue hypoxia, such as living at high altitude, chronic obstructive pulmonary disease, cyanotic heart disease, sleep apnea, high-affinity hemoglobinopathy, smoking, or localized renal hypoxia (1, 2). In other instances, excessive Epo levels are the result of production by neoplastic cells. Cases of increased Epo production and erythrocytosis have been reported for patients with renal carcinomas (3), benign renal tumors (4), Wilms' tumors, hepatomas (5), liver carcinomas (6), cerebellar hemangioblastomas (3, 7, 8), adrenal gland tumors (9), smooth muscle tumors (3, 9), and leiomyomas (10).

Deficient Epo production is found in conjunction with certain forms of anemias. These include anemia of renal failure and end-stage renal disease (1, 2, 11), anemias of chronic disorders such as chronic infections (1), autoimmune diseases (1), rheumatoid arthritis (12), AIDS (13), malignancies (14), anemia of prematurity (2), anemia of hypothyroidism (2), and anemia of malnutrition (2). Many of these conditions are associated with the generation of IL-1 and TNF-α, factors that have been shown to be inhibitors of Epo activity (1, 15). Other forms of anemias, on the other hand, are due to Epo-independent causes and affected individuals show elevated levels of Epo (2). These forms include aplastic anemias, iron deficiency anemias, thalassemias, megaloblastic anemias, pure red cell aplasias, and myelodysplastic syndromes.

The Quantikine™ Human Epo Immunoassay is a 2.5 hour solid phase immunoassay designed to measure human Epo in serum and EDTA plasma. It contains recombinant human Epo, and antibodies raised against the recombinant protein. Natural human Epo showed dose-response curves that were parallel to the standard curves obtained using the Quantikine kit standards, indicating that this kit can be used to determine relative levels of natural human Epo.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Epo has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Epo present is bound by the immobilized antibody. After removing any unbound substances, an enzyme-linked polyclonal antibody specific for human Epo 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 Epo 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 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.

PRECAUTIONS

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DEPRU0B	CATALOG # SEPRU0B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Epo Microplate	899583	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Epo.	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 Epo Conjugate	899584	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human Epo conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Epo Standard 0 mIU/mL	899622	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 2.5 mIU/mL	899623	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 5 mIU/mL	899624	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 20 mIU/mL	899625	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 50 mIU/mL	899626	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 100 mIU/mL	899627	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Human Epo Standard 200 mIU/mL	899628	1 vial	6 vials	2.1 mL of a buffered protein base with preservatives.	
Assay Diluent RD1-136	897211	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-77	896997	1 vial	6 vials	21 mL/vial of a protein stabilized buffer with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
TMB ELISA Substrate	642880	1 vial	6 vials	17 mL/vial of a TMB ELISA Substrate.	
ELISA Stop Solution	642878	1 vial	6 vials	17 mL/vial of a methanesulfonic acid solution.	
Plate Sealers	N/A	4 strips	16 strips	Adhesive strips	

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

DEPRU0B contains sufficient material to run an ELISA on one 96-well plate.

SEPRU0B (SixPak) contains sufficient material to run ELISAs on six 96-well plates.

This kit is also available in a PharmPak (R&D Systems®, # PDEPRU0B). 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 datasheets 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
Human Epo Microplate	899583	50 plates
Human Epo Conjugate	899584	50 vials
Human Epo Standard 0 mIU/mL	899622	25 vials
Human Epo Standard 2.5 mIU/mL	899623	25 vials
Human Epo Standard 5 mIU/mL	899624	25 vials
Human Epo Standard 20 mIU/mL	899625	25 vials
Human Epo Standard 50 mIU/mL	899626	25 vials
Human Epo Standard 100 mIU/mL	899627	25 vials
Human Epo Standard 200 mIU/mL	899628	25 vials
Assay Diluent RD1-136	897211	50 vials
Calibrator Diluent RD5-77	896997	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642880	50 vials
ELISA Stop Solution	642878	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

- Pipettes and pipette tips
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- 500 mL graduated cylinder
- Squirt bottle, manifold dispenser, or automated microplate washer
- Absorbent pad or paper towels for blotting the wells.
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540, 570, or 600 nm
- Deionized or distilled water
- **Polypropylene** test tubes for dilution of samples

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 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.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and EDTA plasma can be tested neat. Some samples may require dilution with Calibrator Diluent RD5-77 due to high endogenous levels. Multiple dilutions are recommended for unknown samples. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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.

Standard - Discrete standards are provided at concentrations indicated on vials.
Seven standard vials are provided; 200 mIU/mL, 100 mIU/mL, 50 mIU/mL, 20 mIU/mL, 5 mIU/mL, 2.5 mIU/mL, and 0 mIU/mL.

ASSAY PROCEDURE

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

1. Prepare all reagents as directed in the previous section.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Pipette 100 µL of Assay Diluent RD1-136 into each well.
4. Add 100 µL of standard or sample per well. Cover the plate 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. A plate layout is provided containing a sample diagram of standards and samples.
5. Thoroughly aspirate or decant the contents from each well. Blot dry on clean paper towels. **Do not wash.**
6. Add 200 µL of Human Epo Conjugate to each well. Cover the plate with a new adhesive strip. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker.
7. Aspirate each well and wash, repeating the process three times for a total of 4 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential for 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.
8. Add 150 µL of TMB ELISA Substrate solution to each well. Incubate for 20-25 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 150 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 (O.D.) of each well within 15 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 600 nm. If wavelength correction is not available, subtract readings at 600 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

❶ Prepare all reagents as instructed.

❷ Add 100 µL of Assay Diluent RD1-136 to each well.

❸ Add 100 µL of standard or sample to each well.
Seal and incubate at RT for 1 hour on a shaker.

❹ Aspirate. **Do not wash.**

❺ Add 200 µL of conjugate to each well.
Seal and incubate at RT for 1 hour on shaker.

❻ Aspirate and wash 4X with Wash Buffer.

❼ Add 150 µL of TMB Substrate to each well.
Incubate at RT **on the benchtop in the dark** for 20-25 minutes.

❽ Add 150 µL of Stop Solution to each well.

❾ Read at 450 nm within 15 minutes with a correction at 600 nm.

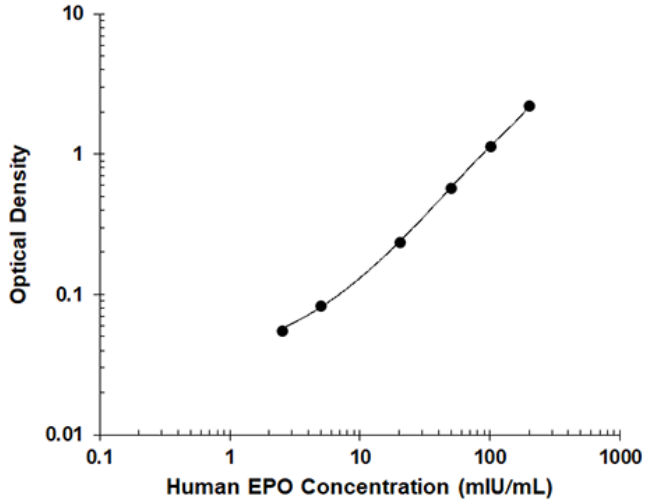
CALCULATION OF RESULTS

Read the absorbance of each well on a microplate reader using 450 nm as the primary wavelength and 600 nm as the reference wavelength (540, 570, or 650 nm is acceptable). Average the duplicate readings for each standard and sample and subtract the average 0 mIU/mL standard optical density (O.D.).

Create a standard curve by reducing the data using 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 Epo 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.

TYPICAL DATA

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



(mIU/mL)	O.D.	Average	Corrected
0	0.010 0.013	0.012	—
2.5	0.054 0.056	0.055	0.043
5.0	0.083 0.084	0.084	0.072
20	0.235 0.237	0.236	0.224
50	0.575 0.583	0.579	0.567
100	1.121 1.152	1.137	1.125
200	2.226 2.226	2.226	2.214

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 (mIU/mL)	10.3	40.3	117	10.4	41.9	118
Standard deviation	0.370	1.45	1.97	0.860	2.20	5.78
CV (%)	3.6	3.6	1.7	8.3	5.3	4.9

RECOVERY

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

Specimen Type	Average % Recovery	Range
Serum (n=4)	101	96-107
EDTA plasma (n=4)	94	84-98

SENSITIVITY

Thirty-three assays were evaluated and the minimum detectable dose (MDD) of Epo ranged from 0.094 – 0.570 mIU/mL. The mean MDD was 0.221 mIU/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 spiked with high concentrations of human Epo in various matrices were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA Plasma (n=4)
1:2	Average % of Expected	108	112
	Range (%)	106-109	106-117
1:4	Average % of Expected	106	110
	Range (%)	94-113	100-119
1:8	Average % of Expected	99	103
	Range (%)	91-106	94-114

CALIBRATION

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

The NIBSC/WHO Human Erythropoietin International Standard 11/170 (recombinant human EPO) was evaluated in this kit. The dose response curve in this International Standard parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine™ Human Epo kit to approximate NIBSC/WHO 11/170 International units, use the equation below.

NIBSC/WHO (11/170) approximate value (mIU/mL) = 1.5877 x Quantikine™ Human Epo value (mIU/mL)

Based on data generated from May 2025.

SAMPLE VALUES

Samples from apparently healthy volunteers were evaluated for the presence of human Epo in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (mIU/mL)	Range (mIU/mL)	Standard Deviation (mIU/mL)
Serum (n=40)	11.1	3.31-38.2	6.25
EDTA plasma (n=40)	10.4	3.16-36.2	6.05

SPECIFICITY

This assay recognizes natural and recombinant human Epo.

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 Epo control were assayed for interference. No cross-reactivity or interference was observed.

Recombinant human:

MAN-1A1
CD-26
3-OST1
CSPG-4
BMP-4
DSC-3
Clusterin
LDLR
SALM-4
Eph B4

Interference of matrix-associated factors was tested by spiking a mid-range Epo control and calibrator diluent with selected amounts of the following substances and analyzing for the presence of Epo in the Quantikine™ Human Epo ELISA. No interference was observed.

Protein	Amount Added
Albumin	3 g/dL
Bilirubin	20 mg/dL
Gamma globulin	5 mg/dL
Hemoglobin	30 mg/dL
Transferrin	200 mg/dL
Triolein	500 mg/dL
α1-Acid Glycoprotein	80 mg/dL
α-2 Macroglobulin	30 mg/dL

The following factors were assayed for cross-reactivity and interference by spiking calibrator diluent and a mid-range Epo control with selected amounts of the following substances and analyzing for the presence of Epo in the Quantikine Human Epo ELISA.

Recombinant Protein	Cross-reactivity	Interference
Canine Epo	1%	>2 ng/mL
Human Epo R	0%	>400 pg/mL
Mouse Epo	15%	>80 pg/mL
Mouse Epo R	0%	>400 pg/mL
Rat Epo	5%	>400 pg/mL

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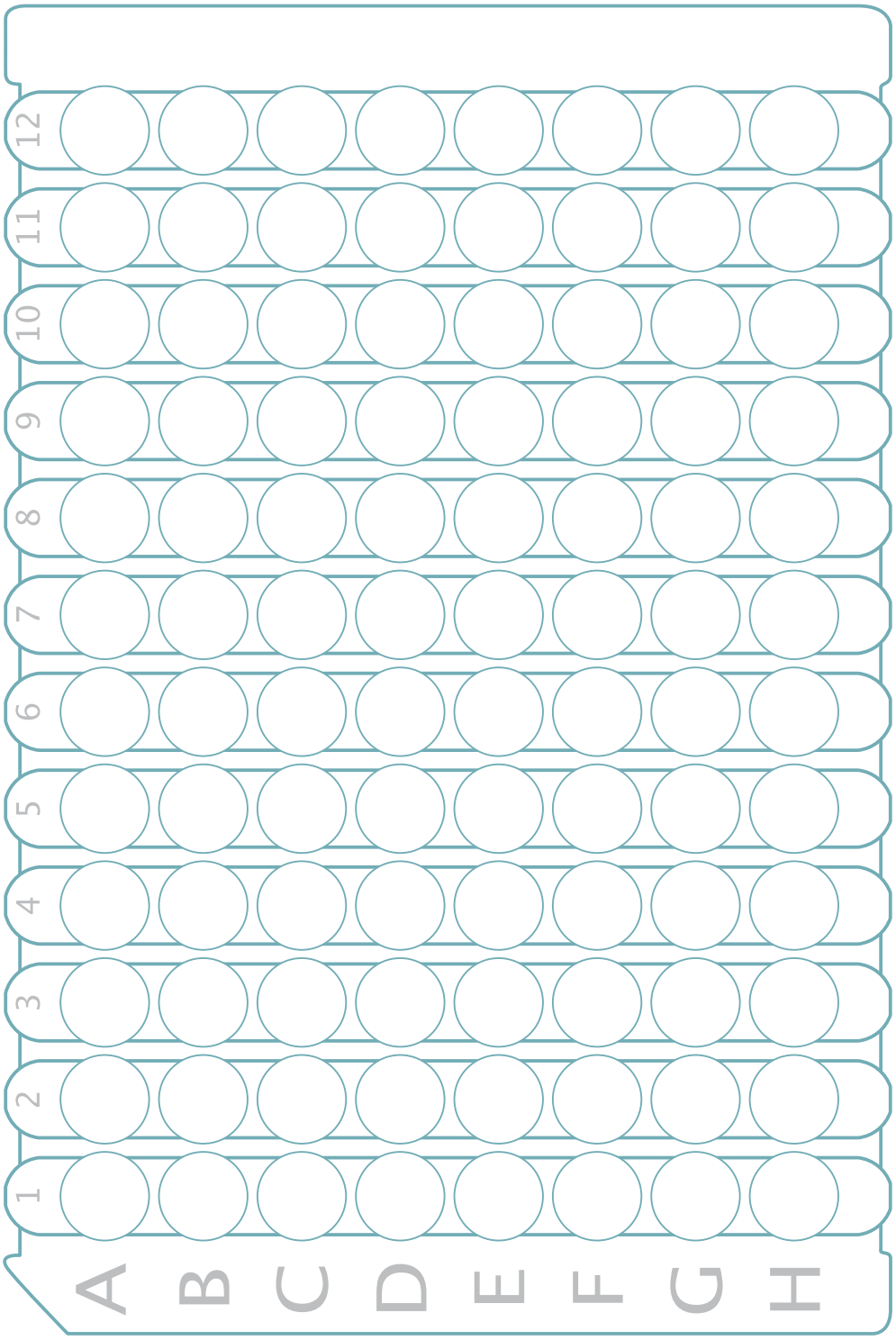
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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