ELISA

HT 8-oxo-dG ELISA Kit II

Catalog Numbers: 4380-096-K
4380-192-K

High throughput ELISA to quantify 8-oxo-dG in DNA, plasma, saliva, and urine samples.

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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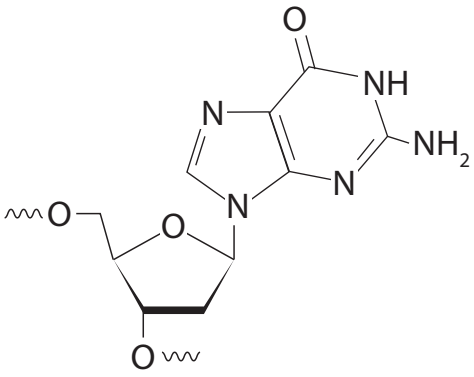
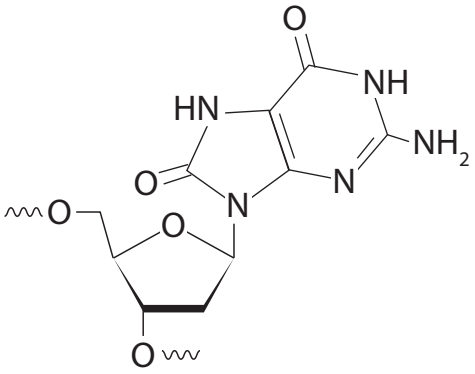
INTRODUCTION

Mutagenic reactive oxygen species (ROS) are implicated in cancer, neurodegenerative disorders such as Alzheimer's disease (1), and in apoptosis (2). When exposed to oxidative radicals, 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) is formed and can serve as a sensitive indicator of physiological and environmental damage to DNA. The production of 8-hydroxyguanine is almost exclusively elicited by oxidative stress with the main attack site by oxidative radicals at the N7-C8 bond. 8-hydroxyguanine is labile, resulting in an abasic lesion whereby DNA polymerase preferentially inserts adenine opposite the abasic site. Therefore, without repair these oxidative damage adducts can lead to G to T transitions (3). The 8-hydroxyguanine lesion causes mutational frequencies of 1-5% (mainly G:C to T:A transitions) and is one of the most abundant oxidative lesions (4). 8-oxo-dG is a frequently used biomarker of oxidative DNA damage and oxidative stress.



deoxyguanosine



8-hydroxy-2'-deoxyguanosine

PRINCIPLE OF THE ASSAY

The HT 8-oxo-dG ELISA Kit II is a fast and sensitive immunoassay for the detection and quantitation of 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) in DNA, plasma, urine, and saliva samples. This assay employs a 96 strip well pre-coated with 8-oxo-dG, an anti-8-oxo-dG monoclonal mouse antibody, a horseradish peroxidase (HRP) conjugated secondary antibody, and colorimetric detection substrate to construct a flexible, high throughput assay. The 8-oxo-dG monoclonal antibody binds competitively to 8-oxo-dG immobilized on pre-coated wells and in solution. Antibody bound to 8-oxo-dG in the sample is washed away while antibody bound to 8-oxo-dG attached to the well is retained. Detection is performed with HRP conjugate and colorimetric substrate. Product formation is inversely proportional to amount of 8-oxo-dG present in sample.

Important features of the assay include: 1) colorimetric, non-radioactive format; 2) high throughput 96 strip wells; 3) dynamic range from 3.13 nM to 200 nM (0.89 ng/mL to 56.7 ng/mL); and, 4) sensitivity at 2 nM (0.57 ng/mL) 8-OHdG.

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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- Although this assay has been validated for use with DNA, plasma, urine, and saliva, some samples may contain higher levels of interfering factors that can compromise the performance of the assay, or produce inaccurate results.
- If samples generate values greater than the 200 nM standard, assay at a higher sample dilution. If samples generate values lower than 3.13 nM standard, assay at a lower sample dilution.

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.

PRECAUTION

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

MATERIALS PROVIDED & STORAGE CONDITIONS

Do not use past expiration date.

PART	PART #	CATALOG # 4380-096-K	CATALOG # 4380-192-K	DESCRIP-TION	STORAGE OF	
					UNOPENED MATERIAL	OPENED/ RECONSTITUTED MATERIAL
8-oxo-dG Microplate	4380-096-P	1 plate	2 plates	96-well polystyrene microplate (12 strips of 8 wells) coated with 8-oxo-dG.	Store 2-8 °C.	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.
Assay Diluent RD1-128	4380-096-02	1 vial	2 vials	50 mL/vial	Store 2-8 °C.	Store 2-8 °C.
TACS-Sapphire	4822-96-08	1 vial	2 vials	10 mL/vial		
100X Cations	4380-096-05	1 vial	2 vials	500 µL/vial		
8-oxo-dG Standard	4380-096-01	1 vial	2 vials	20 µL/vial	Store at ≤ -20 °C.	Store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
8-oxo-dG Monoclonal Antibody	4380-096-03	1 vial	2 vials	20 µL/vial		
Goat anti-Mouse IgG HRP	4380-096-04	1 vial	2 vials	20 µL/vial		
DNase I (5 Units/µL)	4380-096-06	1 vial	2 vials	40 µL/vial		
8-oxo-dG Alkaline Phosphatase (1 Unit/µL)	4380-096-07	1 vial	2 vials	40 µL/vial		
Plate Sealers	N/A	4 Adhesive strips				

OTHER SUPPLIES REQUIRED

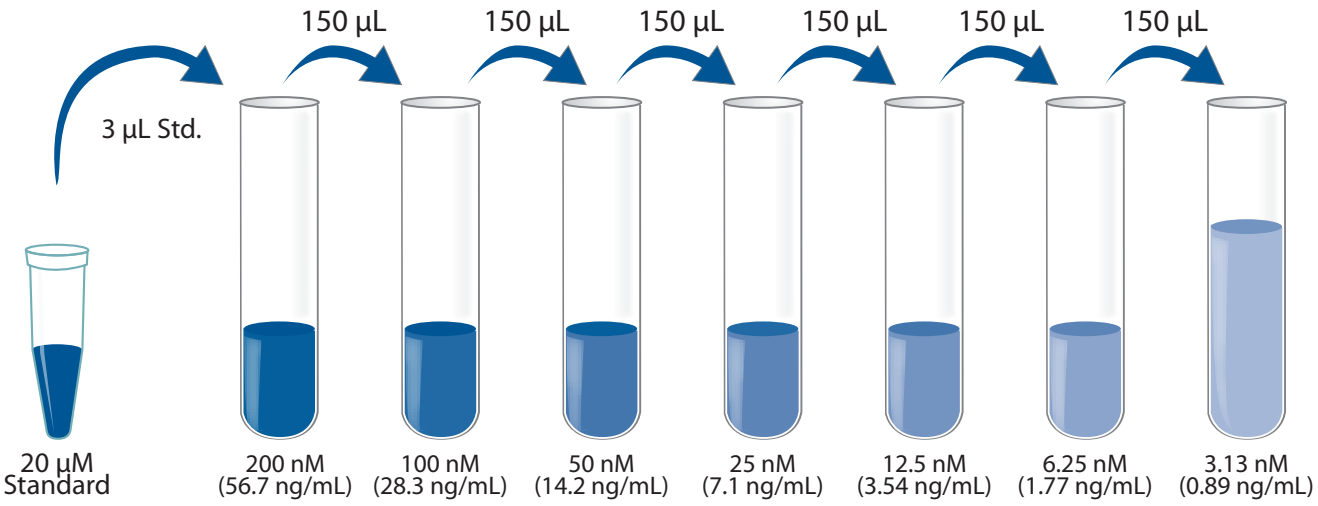
- 1X PBS
- Tween® 20
- 0.2 M HCl or 5% Phosphoric acid
- DNA extraction kit, i.e. QIAGEN® Flexigene® Kit for cultured cells and QIAGEN® PAXgene® kit for tissues
- Pipettes and pipette tips
- Microcentrifuge tubes
- Pipette-aid, pipettor and multichannel pipettor
- Squirt bottle, manifold dispenser, or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Vortex
- Microcentrifuge
- Heat block
- Incubator set at 25 °C

REAGENT PREPARATION

PBS + 0.1% Tween 20 Wash Solution (PBST) - Prepare 500 mL of 1X PBST containing 1X PBS and 0.1% Tween 20 in a squirt bottle for washing strip wells.

8-oxo-dG Standard - The kit contains 20 µL of 8-oxo-dG Standard at a concentration of 20 µM. Centrifuge the standard vial before opening cap. Aliquot and avoid repeated freeze/thaw cycles.

Pipette 297 µL of Assay Diluent RD1-128 into the 200 nM tube. Pipette 150 µL into the remaining tubes. Use the 8-oxo-dG Standard to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 nM standard serves as the high standard. Assay Diluent RD1-128 serves as the zero standard (0 nM).



Anti-8-oxo-dG Monoclonal Antibody Solution - Immediately before use, dilute the 8-oxo-dG Monoclonal Antibody 250-fold with Assay Diluent RD1-128. A total of 25 µL/well of diluted anti-8-oxo-dG Monoclonal Antibody Solution is required in the assay. For 96 wells, dilute 12 µL of anti-8-oxo-dG Monoclonal Antibody into 3 mL of Assay Diluent RD1-128.

Goat Anti-Mouse IgG HRP Conjugate - Immediately before use, dilute Goat anti-Mouse IgG HRP 500-fold with Assay Diluent RD1-128. A total of 50 µL/well of diluted Goat anti-Mouse IgG HRP Conjugate is required in the assay. For 96 wells, dilute 12 µL of Goat anti-Mouse IgG HRP into 6 mL of Assay Diluent RD1-128.

TACS-Sapphire™ - Pre-warm TACS-Sapphire™ to room temperature before use. TACS-Sapphire is a colorimetric substrate that turns blue in the presence of Horseradish Peroxidase (HRP). The addition of an equal volume of 0.2 M HCl or 5% phosphoric acid stops the reaction to generate a yellow color that can be read at 450 nm. A total of 50 µL is required per well.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

DNA Samples:

Cultured Suspension Cells - Grow 1-5 x 10^6 cells in suspension using complete medium in a suitable tissue culture plate or flask. Count the cells. Harvest cells by centrifugation and remove growth medium. Wash one time with 1X PBS. Suspend cell pellets at 1 x 10^6 cells/mL in ice-cold 1X PBS. For example, add 5 mL 1X PBS to 5 x 10^6 cells. Aliquot 1 mL into 1.5 mL microcentrifuge tubes. Centrifuge at 10,000 x g for 10 seconds at 2-8 °C. Discard supernatant. **Proceed to DNA Extraction.** (Cell pellets can be flash frozen in liquid nitrogen and stored at ≤ -70 °C for later use.)

Cultured Adherent Cells - Grow 1-5 x 10^6 adherent cells in complete medium in a suitable tissue culture dish or flask until 75% confluent. Remove the growth medium and harvest cells by trypsinization or a method of choice. Count the cells. Wash one time with 1X PBS. Suspend the cell pellets at 1 x 10^6 cells/mL in ice-cold 1X PBS. For example, add 5 mL 1X PBS to 5 x 10^6 cells. Aliquot 1 mL into 1.5 mL microcentrifuge tubes. Centrifuge at 10,000 x g for 10 seconds at 2-8 °C. Discard supernatant. **Proceed to DNA Extraction.** (Cell pellets can also be flash frozen in liquid nitrogen and stored at ≤ -70 °C for later use).

Tissue Samples - The tissue specimen should be cut into a 2 mm cube and weigh approximately 8-12 mg in mass. **Proceed to DNA Extraction.**

DNA Extraction - Extract DNA from the above cultured cells or tissue samples by a desired method or commercial extraction kit. (Generally the minimal amount of extracted DNA required for each sample is 20-50 µg.) Quantitate DNA spectrophotometrically (OD_{260} = 50 µg/mL). The suggested final DNA concentration is 200 µg/mL to 1000 µg/mL. Add 100X Cations to DNA solution for final 1X concentration. Add 2 µL DNase I per 50 µg DNA and incubate for 1 hour at 37 °C. Add 2 µL Alkaline Phosphatase per 50 µg DNA and incubate 1 hour at 37 °C. Assay immediately or aliquot and store at ≤ -20 °C.

Plasma Samples - Withdraw blood according to standard procedures using Sodium Heparin or EDTA as anticoagulant. Collect plasma by centrifugation at room temperature in a horizontal rotor (swinging bucket) in a proper adaptor for 15 minutes at 1,500 x g within 30 minutes of blood collection. Carefully transfer the plasma to a new centrifuge tube and centrifuge for another 15 minutes at 2500 x g at room temperature. Carefully transfer the supernatant and assay immediately or store at ≤ -20 °C in aliquots for later use. Avoid repeated freeze-thaw cycles.

SAMPLE COLLECTION & STORAGE *CONTINUED*

Urine Samples - Collect urine according to standard procedure into a sterile container. To clarify, centrifuge 2,000 x g for 15 minutes, or filter using a 0.45 µm filter to remove precipitate. Assay immediately or store at ≤ -20 °C in aliquots for later use. Avoid repeated freeze-thaw cycles.

Saliva Samples - Collect saliva according to standard procedure in a centrifuge tube. To clarify, centrifuge at 2,000 x g for 15 minutes. Carefully remove supernatant and assay immediately or store at ≤ -20 °C in aliquots for later use. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Extracted DNA samples can be directly applied for measurement.

Clarified plasma, saliva, and urine samples require a 10-fold dilution. A suggested 10-fold dilution is 10 µL of sample + 90 µL of Assay Diluent RD1-128.

ASSAY PROTOCOL

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 25 µL of 8-oxo-dG Standards and clarified samples to appropriate wells. Add 25 µL of Assay Diluent RD1-128 to 0 nm 8-oxo-dG and blank wells (background control).
4. Add 25 µL of anti-8-oxo-dG Monoclonal Antibody Solution to all wells except blank wells. Add 25 µL of Assay Diluent RD1-128 to blank wells instead. Mix thoroughly without causing air bubbles. Cover wells with film sealer and incubate at room temperature for 1 hour.
5. Gently remove film sealer, aspirate each well and wash wells 4 times with PBST (300 µL/well). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining PBST by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
6. Add 50 µL of Goat anti-Mouse IgG HRP Conjugate to all wells except blank wells. Add 50 µL of Assay Diluent RD1-128 to blank wells. Cover wells with film sealer and incubate at room temperature for 1 hour. Place TACS-Sapphire™ at 25 °C to pre-warm.
7. Repeat the aspiration/wash as in step 5.
8. Add 50 µL of pre-warmed TACS-Sapphire™ colorimetric substrate to all wells and incubate in the dark, for 15 minutes at room temperature. Stop the reactions by adding 50 µL 0.2 M HCl or 5% Phosphoric Acid to all wells mixing well. Immediately read the absorbance at 450 nm.

CALCULATION OF RESULTS

Average the triplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Plot the log of 8-oxo-dG Standard concentrations (nM) on the X-axis versus relative absorbance on the Y-axis.

The standard curve is a 2nd order polynomial function represented by the equation: $y = a + bx + cx^2$, where y is the relative absorbance, x is the log of 8-oxo-dG concentration in nM and a, b and c are coefficients. Calculate the 8-oxo-dG sample concentrations using the polynomial equation or interpolation from the standard curve.

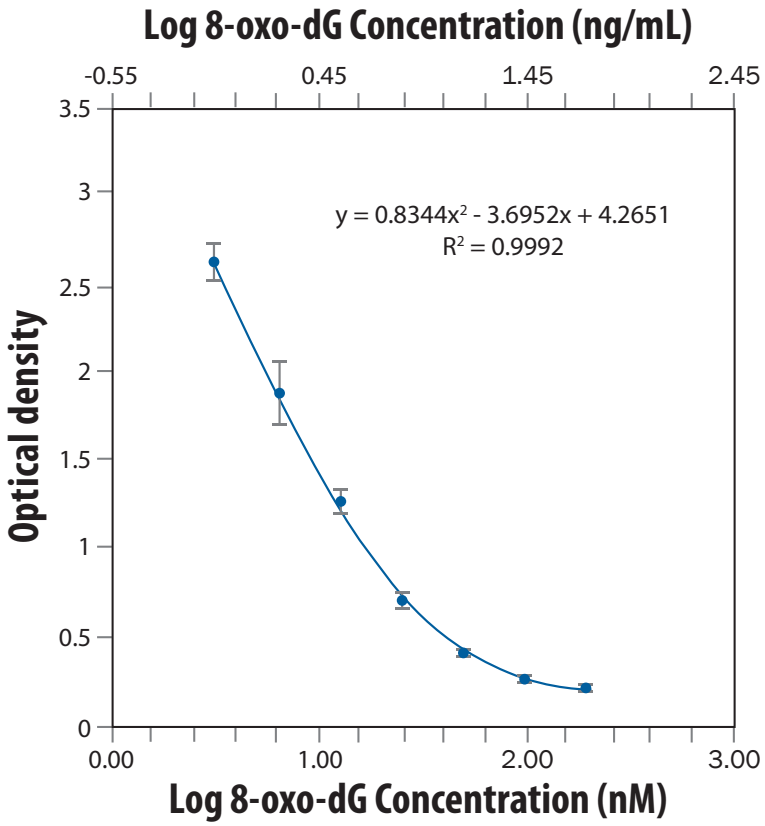
If desired, % B/B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

Calculate the concentration of 8-oxo-dG corresponding to the mean absorbance from the standard curve.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

STANDARD CURVE

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



PERFORMANCE CHARATERISTICS

Intra-Assay Precision: Three samples of known concentration were tested nine times in a single assay. The overall intra-assay coefficient of variation was calculated to be < 10%.

Inter-Assay Precision: Three samples of known concentration were tested nine times in three separate assays. The overall inter-assay coefficient of variation was calculated to be < 15%.

Sensitivity: The LLD (low limit of detection) of 8-oxo-dG was calculated to be 2 nM (0.57 ng/mL).

Spike and Recovery: Test samples were spiked with three different levels of 8-oxo-dG and analyzed for recovery before and after spiking. The calculated overall mean was between 80-120%.

Dilution Linearity: Test samples were serially diluted in Assay Diluent RD1-128 and subsequently measured by the assay. Dilution recovery was assessed by comparing observed vs. expected values based on undiluted samples. The calculated overall mean was between 80-120%.

Specificity: Cross-reactivity of the anti-8-oxo-dG Monoclonal Antibodies with eight analogues of 8-oxo-dG was tested at 50% binding. 8-hydroxyguanosine and 8-hydroxyguanine have a significant cross-reaction. The cross-reactivity reports are as follows:

Cross-Reactant	% Inhibition at IC_{50}
8-Hydroxydeoxyguanosine	100
8-Hydroxyguanosine	~30
8-Hydroxyguanine	~20
8-Mercatoguanosine	~4
8-Bromoguanosine CMP	<0.01
Guanosine	<0.01
Guanine	<0.01
2'-Deoxyinosine	<0.01
N2-Methylguanosine	<0.01

REFERENCES

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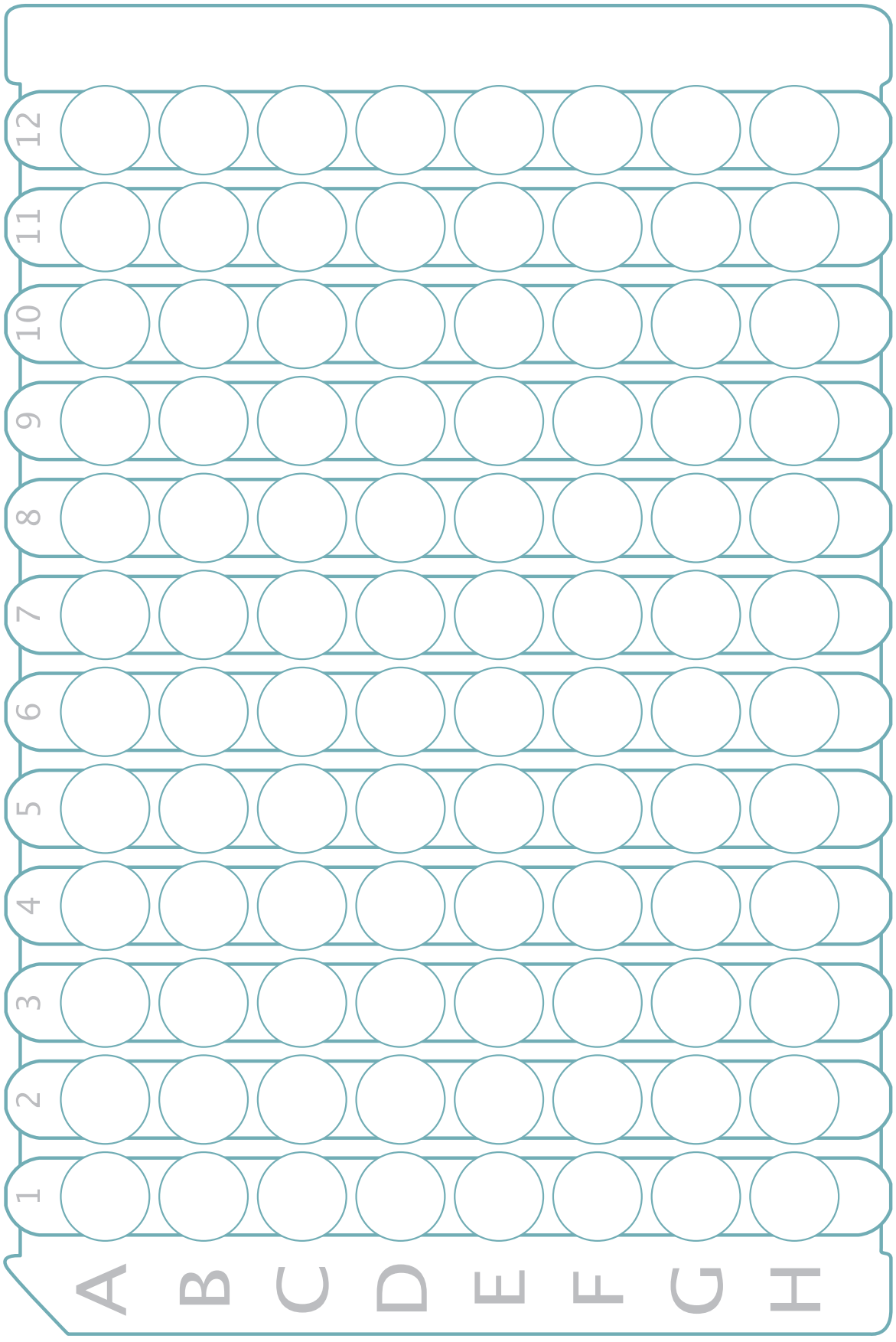
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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