Human Zika Virus IgG ELISA Kit

Catalog Number ZIK00

For the determination of Zika Virus IgG antibody in human serum.

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

Zika virus (ZIKV) is a mosquito-borne flavivirus found throughout tropical and subtropical regions, including East Africa, Southeast Asia, and the Pacific Islands, that is now causing large-scale outbreaks in the Americas (1, 2). This continuous geographic expansion of ZIKV poses a serious and increasing public health threat around the globe (1-4). Initially, ZIKV infection was thought to cause only mild illness, however it has now been linked to a rising number of severe neurological abnormalities and diseases including microcephaly, congenital abnormalities, and nonfetal illnesses such as Guillain-Barré syndrome, which emphasizes the importance of accurate ZIKV diagnostics (2, 4-6). Serological diagnosis is complicated by cross-reactivity among members of the Flavivirus genus (6). Because ZIKV, Dengue virus (DENV), and related flaviviruses, co-circulate in endemic regions and share high sequence similarity, there is a high possibility of IgM and IgG cross-reactivity in immunoassays (7). Current or past infections will often cause false positives requiring the need for follow-up testing and confirmation by a plaque-reduction neutralization (PRNT) assay. PRNT is a complicated method that takes considerable time and has limited availability (7, 8). In addition, antibodies present from past infection by Zika or other flaviviruses may enhance the risk of future ZIKV infections through antibody-dependent enhancement (ADE), which may lead to increased disease severity (9). There is a need for a simple serological test that displays high Zika specificity with minimal cross-reactivity with other flaviviruses.

The Human Zika Virus IgG ELISA Kit is a 3.5 hour solid phase ELISA designed to measure Zika Virus IgG antibody in human serum.

PRINCIPLE OF THE ASSAY

This assay is an antigen-down enzyme immunoassay where a recombinant Zika virus NS1 antigen is pre-coated onto a 96-well microplate and used to bind antibodies found in the sample. When the sample is added (such as human serum), antibodies found in the sample that recognize Zika virus NS1 antigen bind the antigen coated plate and are retained in the well. After washing away unbound substances, an enzyme linked polyclonal antibody specific for human IgG is added to the wells. Following a wash to remove any unbound enzyme linked antibody, a substrate is added to the wells and color develops in proportion to the amount of IgG antibodies in the sample bound to the Zika virus NS1 antigen. The color development is stopped and the intensity of the color is measured.

The potential for false positives due to Zika virus NS1 antigen cross-reactive antibodies to related flaviviruses, such as Dengue virus, is minimized by treatment of the samples. Samples are treated with a proprietary treatment reagent prior to being added to the Zika virus NS1 antigen coated plate. Sample specific background is determined by adding identically treated samples to an uncoated background plate and measuring the amount of IgG antibodies non-specifically bound to the well. To interpret results, net sample readings are calculated by subtracting each sample background plate reading from the Zika virus NS1 antigen plate reading.

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.
- 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 immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- **DO NOT STACK PLATES DURING THE ASSAY. PLATES MUST BE KEPT FLAT.**
- When mixing or reconstituting solutions, avoid excessive foaming.
- To avoid cross-contamination, change pipette tips between control additions, 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Zika Virus IgG Microplate	898864	96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant Zika Virus NS1 antigen.	**Note:** *Immediately after opening the plate bag, mark each plate with the appropriate colored marker (see page 5) to distinguish between plates.* 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.*
Background Plate	898871	96 well polystyrene microplate (12 strips of 8 wells) blocked with BSA.	
Treatment Plate	898887	96 well polystyrene microplate (12 strips of 8 wells).	
Zika Virus IgG Conjugate	898865	21 mL of a polyclonal antibody specific for human IgG conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Positive Control	898866	1 vial of a monoclonal antibody specific for Zika NS1; lyophilized.**	
Negative Control	898867	1 vial of human plasma in a buffered solution with preservatives; lyophilized.**	
Treatment Control	898868	1 vial of flavivirus antibody; lyophilized.**	
Sample Dilution Buffer	896200	5 vials (21 mL/vial) of a buffered protein base with preservatives and blue dye.	
Treatment Reagent	896201	1 vial of a buffered protein base with preservatives; lyophilized.	
Treatment Diluent	896348	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895926	11 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	12 adhesive strips.	

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

**Controls contain human plasma. See Precautions section on page 4.

OTHER SUPPLIES REQUIRED

- Black, blue, and red permanent markers.
- 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.
- 25 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of samples.

PRECAUTIONS

Some components in this kit contain human source materials and have been tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. Because no test method can offer complete assurance that infectious agents are absent, material should be handled as potentially infectious, following precautions as specified in the OSHA Bloodborne Pathogen Rule (29 CFR Part 1910, 1030) or other equivalent biosafety procedures.

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 MSDS 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.

PLATE PREPARATION

Bring all plates to room temperature before use.

Note: *Do not stack plates. Spread out as a single layer. This is important for even temperature distribution. Immediately after opening the plate bag, mark each plate with the appropriate color marker to distinguish between plates.*

Human Zika Virus IgG Microplate - Mark each strip with a **red** permanent marker.

Background Microplate - Mark each strip with a **blue** permanent marker.

Treatment Microplate - Mark each strip with a **black** permanent marker.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum samples require a 100-fold dilution due to endogenous levels. A suggested 100-fold dilution can be achieved by adding 10 µL of untreated sample to 990 µL of Sample Dilution Buffer.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Treatment Reagent - Reconstitute the Treatment Reagent with 14 mL of Treatment Diluent to prepare Treatment Reagent. Reconstitute for a minimum of 15 minutes prior to assay.*

Positive Control - Reconstitute the Positive Control with 1.1 mL of Sample Dilution Buffer to prepare Positive Control. Reconstitute for a minimum of 15 minutes prior to assay.*

Negative Control - Reconstitute the Negative Control with 1.1 mL of Sample Dilution Buffer to prepare Negative Control. Reconstitute for a minimum of 15 minutes prior to assay.*

Treatment Control - Reconstitute the Treatment Control with 1.1 mL of Sample Dilution Buffer to prepare Treatment Control. Reconstitute for a minimum of 15 minutes prior to assay.*

Wash Buffer (1X) - 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 (1X).

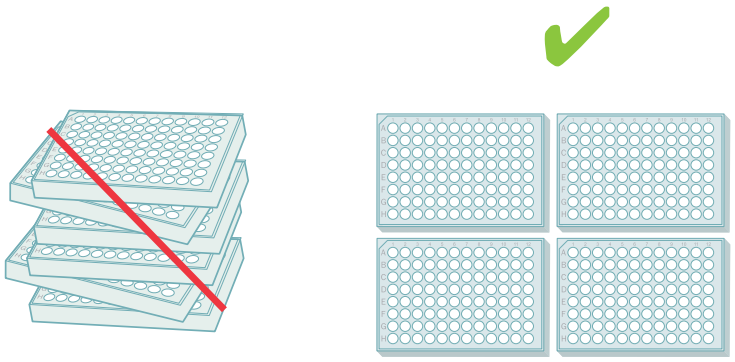
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

*Reconstitute controls and Treatment Reagent with brief gentle inversion. Do not vortex or use constant rotation.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

Note: *Do not stack plates. Spread out as a single layer. This is important for even temperature distribution.*



Sample & Control Treatment (used for Treatment Plate)

1. Dilute serum samples 100-fold in Sample Dilution Buffer (See Sample Preparation).
   Note: *Do not dilute reconstituted controls.*
2. To the Treatment Plate, add 125 µL reconstituted Treatment Reagent to each well.
3. Add 125 µL per well of diluted serum samples or reconstituted controls to each well in duplicate wells for each sample or control. Add 125 µL Sample Dilution Buffer only per well in duplicate wells for non-specific binding (NSB). Cover with adhesive strip. Two plate layout examples are provided. See Plate Layout A on page 7 for separate Human Zika Virus IgG Plate and Background Plate (44 samples). See Plate Layout B on page 8 for Human Zika Virus IgG and Background strips combined on the same plate frame (40 samples).
4. Incubate for 1 hour at room temperature.

Zika Virus IgG Detection (used for both Human Zika Virus IgG & Background plates)

1. From each well of the Treatment Plate: Transfer 100 µL to the Human Zika Virus IgG Microplate and 100 µL to the Background Microplate. Change to a new tip for each well. Cover with adhesive strip.
2. Incubate for 1 hour at room temperature.
3. Aspirate each well and wash 3 times with Wash Buffer (1X) (~400 µL/well). After the last wash, invert plate and blot on clean paper towels.
4. Add 100 µL of Human Zika Virus IgG Conjugate to each well. Cover with adhesive strip.
5. Incubate for 1 hour at room temperature.
6. Aspirate each well and wash 3 times with Wash Buffer (1X) (~400 µL/well). After the last wash, invert plate and blot on clean paper towels.
7. Add 100 µL of Substrate Solution to each well. Protect from light.
8. Incubate for 20 minutes at room temperature.
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 OD of each well within 15 minutes at 450 nm with a correction wavelength at 540 or 570 nm.
11. Calculate each sample and control net OD:
    Net OD = (Average OD from Zika Virus IgG Plate – Average OD of NSB from Zika Virus IgG Plate) – (Average OD from Background Plate – Average OD of NSB from Background Plate)

PLATE LAYOUT A

An example plate layout shown with a method for screening 44 samples plus controls on separate Human Zika Virus IgG and Background plates.

Human Zika Virus IgG Plate (red marker)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample # 1		Sample # 9		Sample # 17		Sample # 25		Sample # 33		Sample # 41	
B	Sample # 2		Sample # 10		Sample # 18		Sample # 26		Sample # 34		Sample # 42	
C	Sample # 3		Sample # 11		Sample # 19		Sample # 27		Sample # 35		Sample # 43	
D	Sample # 4		Sample # 12		Sample # 20		Sample # 28		Sample # 36		Sample # 44	
E	Sample # 5		Sample # 13		Sample # 21		Sample # 29		Sample # 37		Positive Control	
F	Sample # 6		Sample # 14		Sample # 22		Sample # 30		Sample # 38		Negative Control	
G	Sample # 7		Sample # 15		Sample # 23		Sample # 31		Sample # 39		Treatment Control	
H	Sample # 8		Sample # 16		Sample # 24		Sample # 32		Sample # 40		NSB	
	Human Zika Virus IgG Plate (ZVP)											

Background Plate (blue marker)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample # 1		Sample # 9		Sample # 17		Sample # 25		Sample # 33		Sample # 41	
B	Sample # 2		Sample # 10		Sample # 18		Sample # 26		Sample # 34		Sample # 42	
C	Sample # 3		Sample # 11		Sample # 19		Sample # 27		Sample # 35		Sample # 43	
D	Sample # 4		Sample # 12		Sample # 20		Sample # 28		Sample # 36		Sample # 44	
E	Sample # 5		Sample # 13		Sample # 21		Sample # 29		Sample # 37		Positive Control	
F	Sample # 6		Sample # 14		Sample # 22		Sample # 30		Sample # 38		Negative Control	
G	Sample # 7		Sample # 15		Sample # 23		Sample # 31		Sample # 39		Treatment Control	
H	Sample # 8		Sample # 16		Sample # 24		Sample # 32		Sample # 40		NSB	
	Background Plate (BGP)											

PLATE LAYOUT B

An example plate layout shown with a method for screening 40 samples plus controls.

Human Zika Virus IgG (red marker) and Background (blue marker) strips combined on same plate frame

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample # 1				Sample # 9				Sample # 17			
B	Sample # 2				Sample # 10				Sample # 18			
C	Sample # 3				Sample # 11				Sample # 19			
D	Sample # 4				Sample # 12				Sample # 20			
E	Sample # 5				Sample # 13				Positive Control			
F	Sample # 6				Sample # 14				Negative Control			
G	Sample # 7				Sample # 15				Treatment Control			
H	Sample # 8				Sample # 16				NSB			
	ZVP		BGP		ZVP		BGP		ZVP		BGP	

Human Zika Virus IgG (red marker) and Background (blue marker) strips combined on same plate frame

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample # 21				Sample # 29				Sample # 37			
B	Sample # 22				Sample # 30				Sample # 38			
C	Sample # 23				Sample # 31				Sample # 39			
D	Sample # 24				Sample # 32				Sample # 40			
E	Sample # 25				Sample # 33				Positive Control			
F	Sample # 26				Sample # 34				Negative Control			
G	Sample # 27				Sample # 35				Treatment Control			
H	Sample # 28				Sample # 36				NSB			
	ZVP		BGP		ZVP		BGP		ZVP		BGP	

QUALITY CONTROL

For a valid assay, NSB, Negative Control, Treatment Control, and Positive Control should fall in the net optical density (O.D.) values below.

Calculated Net O.D. Values	Control Result for Valid Assay
< 0.100	NSB, Negative Control, and Treatment Control
≥ 0.400	Positive Control

INTERPRETATION OF RESULTS

The cut-off was selected using values from a small set of field data and is an estimate only. Suggested data interpretation is included in the table below.

Calculated Net O.D. Values	Result	Interpretation
< 0.100	Negative	No detectable Zika Virus IgG antibody.
0.100-0.200	Equivocal	Sample is suspect for Zika Virus IgG antibody. Repeat the test. If sample gives a similar O.D., then Zika Virus IgG antibody cannot be determined, and testing should be repeated by an alternative method or another sample should be collected.
> 0.200	Positive	Zika Virus IgG antibody detected.

PRECISION

Intra-assay Precision (Precision within an assay)
Three Zika Virus IgG positive samples with high, middle, and low net O.D. were tested twenty-four times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three Zika Virus IgG positive samples with high, middle, and low net O.D. were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	24	24	24	20	20	20
Net O.D.	0.597	1.08	1.94	0.616	0.992	1.81
Standard deviation	0.021	0.056	0.139	0.058	0.113	0.168
CV (%)	3.5	5.2	7.2	9.4	11.4	9.3

SPECIFICITY

This assay recognizes Zika Virus specific human IgG antibodies with minimal cross-reactivity of human Dengue Virus IgG antibodies.

Zika Samples

Zika Virus IgG ELISA	# Zika Samples Tested[a]	Positive	Negative	Equivocal	% Positive
R&D Systems®	50	46	3[b]	1	92
Competitor A	50	50	0	NA	100
Competitor E	50	50	0	0	100
Competitor M	50	2	48	NA	4

Dengue Samples

Zika Virus IgG ELISA	# Dengue Samples Tested[c]	Positive	Negative	Equivocal	% Positive
R&D Systems®	9	0	9	0	0
Competitor A	9	6	3	NA	66.7
Competitor E	9	4	5	0	44.4
Competitor M	9	0	9	NA	0

Healthy Donor Samples

Zika Virus IgG ELISA	# Healthy Donor Samples	Positive	Negative	Equivocal	% Positive
R&D Systems®	5	0	5	0	0
Competitor A	5	2	3	NA	40
Competitor E	5	0	5	0	0
Competitor M	5	0	5	NA	0

[a]Zika patient samples were collected from Columbia between 2015 and 2016 and determined to be positive based on EIA, testing according to the sample supplier.

[b]When tested without Treatment Reagent, these samples test positive for Zika Virus IgG, but are negative following Treatment Reagent treatment, indicating competitors A & E positive results from these samples are false positives.

[c]Dengue patient samples were collected from Puerto Rico between 2012 and 2013 before Zika was introduced into Puerto Rico. On December 31, 2015, the United States reported the first PCR-confirmed case of locally acquired Zika infection in Puerto Rico. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5034643/; http://www.who.int/bulletin/online_first/16-171082/en/

Competitor A, E, and M kits have either high cross-reactivity with Dengue Virus IgG or low sensitivity to Zika Virus IgG. R&D Systems® Zika Virus IgG ELISA kit has high sensitivity and specificity to Zika Virus IgG.

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9. Paul, L.M. *et al.* (2016) Clin. Transl. Immunol. **5**:e117.

PLATE LAYOUT

Use this plate layout to record controls and samples assayed.

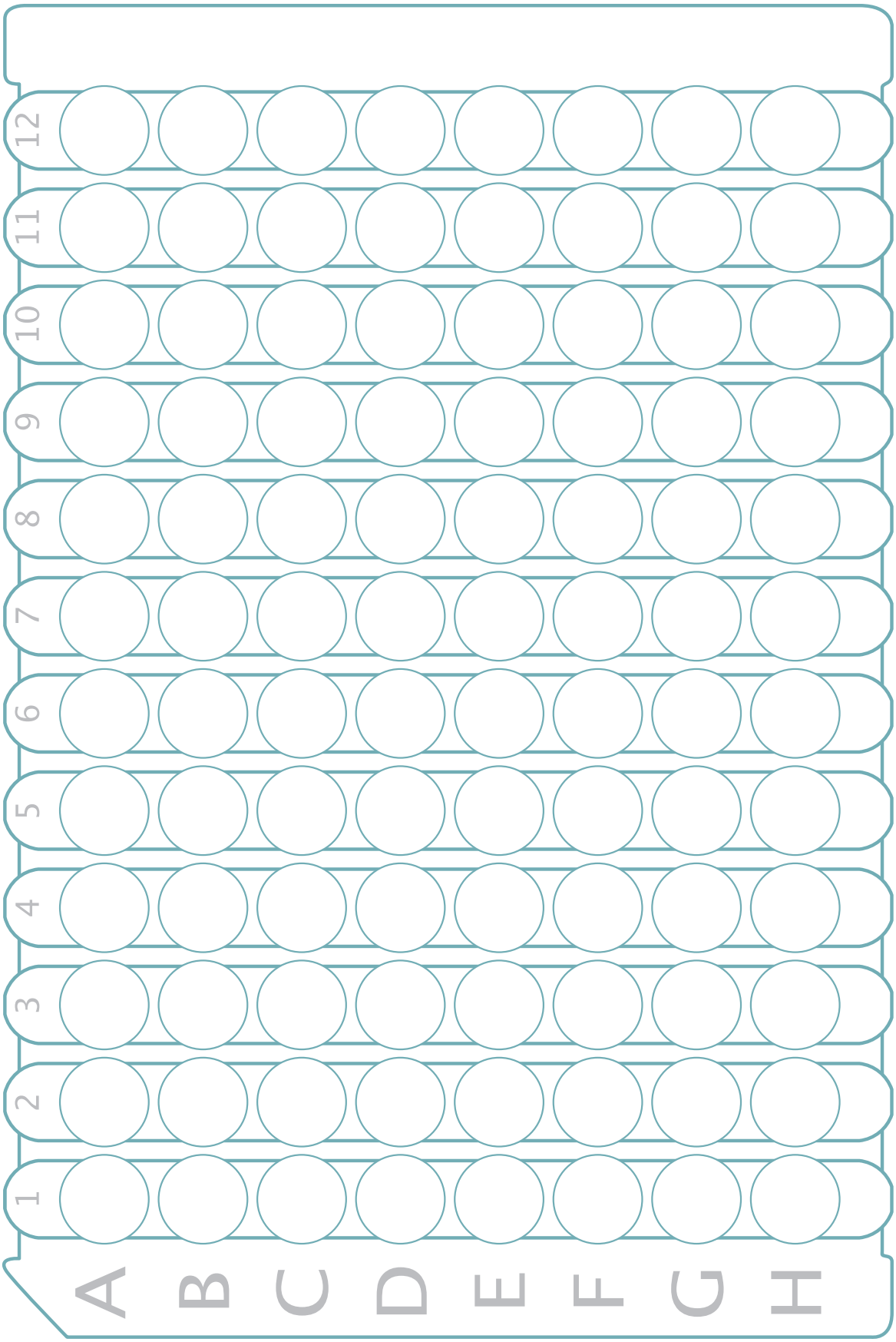
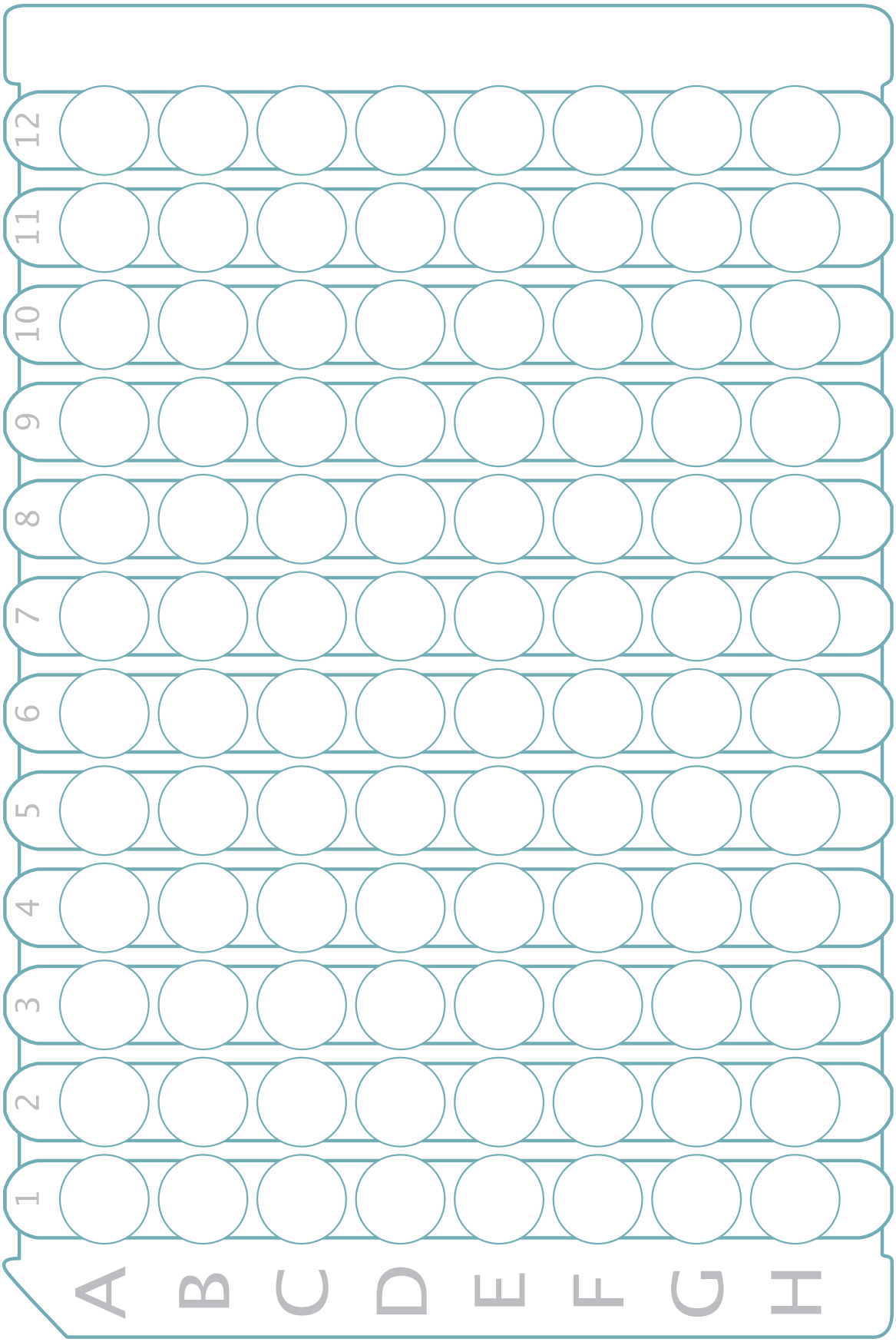


PLATE LAYOUT

Use this plate layout to record controls and samples assayed.



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Use this plate layout to record controls and samples assayed.