Human Anti-Dengue Virus IgG ELISA Kit

Catalog Number DENG00

For the determination of anti-Dengue Virus IgG antibody in human serum or 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

Dengue fever is a mosquito-borne tropical disease caused by the Dengue Virus (DENV), a member of the flavivirus genus. Symptoms of Dengue fever include a high fever, headache, vomiting, muscle and joint pains and a characteristic skin rash. In a small proportion of cases, the disease develops into the life-threatening Dengue hemorrhagic fever, which results in bleeding, low levels of blood platelets and blood plasma leakage, or into Dengue shock syndrome, where dangerously low blood pressure occurs (1). The incidence of Dengue Virus infections has grown dramatically around the world in recent decades. The actual numbers of Dengue cases are underreported, and many cases are misclassified. One recent estimate indicates 390 million Dengue infections per year of which 96 million manifest a level of disease severity (2). Serological diagnosis of Dengue infection is complicated by cross-reactivity among other flaviviruses, such as Zika Virus (ZIKV) (3). Because DENV, ZIKV, and other flavivirus co-circulate in endemic regions and share high sequence similarity, there is a high possibility of IgM and IgG cross-reactivity in immunoassays (4). There is a need for a simple serological test that displays high Dengue specificity with minimal cross-reactivity with other flaviviruses.

The Human Anti-Dengue Virus IgG ELISA Kit is a 3.5 hour solid phase ELISA designed to measure Dengue Virus IgG antibody in human serum and EDTA plasma.

PRINCIPLE OF THE ASSAY

This is an antigen-down enzyme immunoassay where recombinant Dengue Virus strains 1, 2, 3, & 4 NS1 antigens are 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 or EDTA plasma), antibodies found in the sample that recognize Dengue Virus NS1 antigens 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 Dengue Virus NS1 antigens. The color development is stopped, and the intensity of the color is measured.

The potential for false positives due to Dengue Virus NS1 antigen cross-reactive antibodies to related flaviviruses, such as Zika Virus, is minimized by treatment of the samples. Samples are treated with a propriety treatment reagent prior to being added to the Dengue 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 OD readings are calculated by subtracting each sample background plate reading from the Dengue 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. SPREAD OUT AS A SINGLE LAYER.**
- 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 Dengue Virus IgG Microplate	899022	96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant Dengue Virus strains 1, 2, 3, and 4 NS1 antigen and blocked.	**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	899078	96 well polystyrene microplate (12 strips of 8 wells) blocked.	
Treatment Plate	898887	96 well polystyrene microplate (12 strips of 8 wells).	
Anti-human Dengue Virus IgG Conjugate	899023	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	899025	1 vial of a monoclonal antibody specific for Dengue NS1; lyophilized.**	
Negative Control	899026	1 vial of flavivirus antibody-negative human sample in a buffered solution with preservatives; lyophilized.**	
Treatment Control	899027	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	899024	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 source material. 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 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 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: *Heparin and Citrate plasma have not been validated in this assay.*

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 Dengue 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 or EDTA plasma samples require a 50-fold dilution due to endogenous levels. A suggested 50-fold dilution can be achieved by adding 10 µL of untreated sample to 490 µL of Sample Dilution Buffer.
Note: *Samples are diluted 50-fold in Sample Dilution Buffer, then diluted 2-fold in Treatment Reagent for a final dilution of 100-fold.*

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).

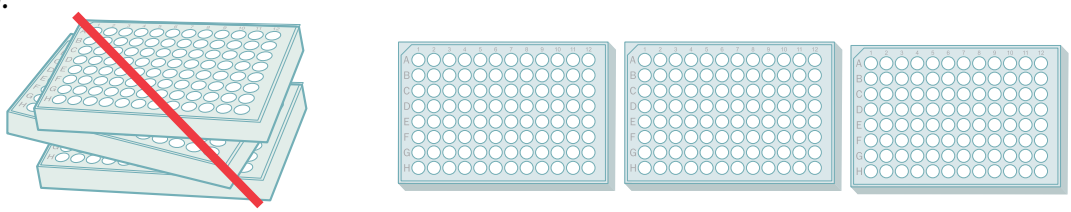
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

*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)

Note: *Two plate layout examples are provided. If testing a full plate, see Plate Layout A on page 7 for separate Human Dengue Virus IgG Plate and Background Plate (44 samples). If testing a partial plate, see Plate Layout B on page 8 for Human Dengue Virus IgG and Background strips combined on the same plate frame (up to 40 samples).*

1. Dilute serum or EDTA plasma samples 50-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 of diluted serum or EDTA plasma samples or reconstituted controls to each well in duplicate wells for each sample or control. Add 125 µL of Sample Dilution Buffer only per well in duplicate wells for non-specific binding (NSB). Cover with an adhesive strip.
4. Incubate for 1 hour at room temperature.

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

1. From each well of the Treatment Plate: Transfer 100 µL to the Human Dengue 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 Dengue 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 average (Avg) OD:
    Avg OD = Average OD from Dengue Virus IgG Plate OR Average OD from Background Plate
12. Calculate each sample and control net OD:
    Net OD = (Average OD from Dengue Virus IgG Plate – Average OD of NSB from Dengue 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 Dengue Virus IgG and Background plates.

Treatment Plate (black 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	
	Treatment Plate											

Human Dengue 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 Dengue Virus IgG Plate (DVP)											

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 up to 40 samples plus controls.

Treatment Plate (black marker)

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

Human Dengue 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			
	DVP		BGP		DVP		BGP		DVP		BGP	

Human Dengue 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			
	DVP		BGP		DVP		BGP		DVP		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
< 0.170	Negative Control and Treatment Control
≥ 0.500	Positive Control

INTERPRETATION OF RESULTS

The cut-offs were selected using values from a small set of field data and are estimates only. Suggested data interpretation is included in the table below.

Calculated Net O.D. Values	Result	Interpretation
< 0.170	Negative	No detectable Dengue Virus IgG antibody.
0.170-0.260	Equivocal	Sample is suspect for Dengue Virus IgG antibody. Repeat the test. If sample gives a similar O.D., then Dengue Virus IgG antibody cannot be determined, and testing should be repeated by an alternative method or another sample should be collected.
> 0.260	Positive	Dengue Virus IgG antibody detected.

SAMPLE TESTING DATA

This assay recognizes Dengue Virus specific human IgG antibodies with minimal cross-reactivity with human Zika Virus IgG antibodies. See Figure 1 and 2 for supporting data.

Anti-Dengue Virus IgG ELISA	# of Samples Tested	Positive		Negative		Equivocal	% Positive	
Dengue Samples[a]	34	30		4		0	88	
Zika Samples[b]	20	18*	20**	2*	0**	0	90*	100**
Healthy Donor Samples[c]	34	0		34		0	0	

*Assay followed Anti-Dengue Virus IgG ELISA kit instructions.

**All samples were tested without sample pre-treatment (without Treatment Reagent).

[a]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. Samples were determined to be Dengue Virus IgG positive by the sample supplier.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5034643/;
http://www.who.int/bulletin/online_first/16-171082/en/

[b]Zika patient samples were collected from Colombia between 2015 and 2016 and determined to be Zika Virus IgG positive by the sample supplier. Several documented outbreaks of Dengue Virus have occurred in Columbia since 1990. There exists the possibility that samples collected in Columbia may be double-positive for both Dengue and Zika Virus IgGs.

[c]Samples from apparently healthy donors were evaluated for the presence of Dengue Virus IgG in this assay. No medical histories were available for the donors used in this study.

SAMPLE TESTING DATA *CONTINUED*

Figure 1

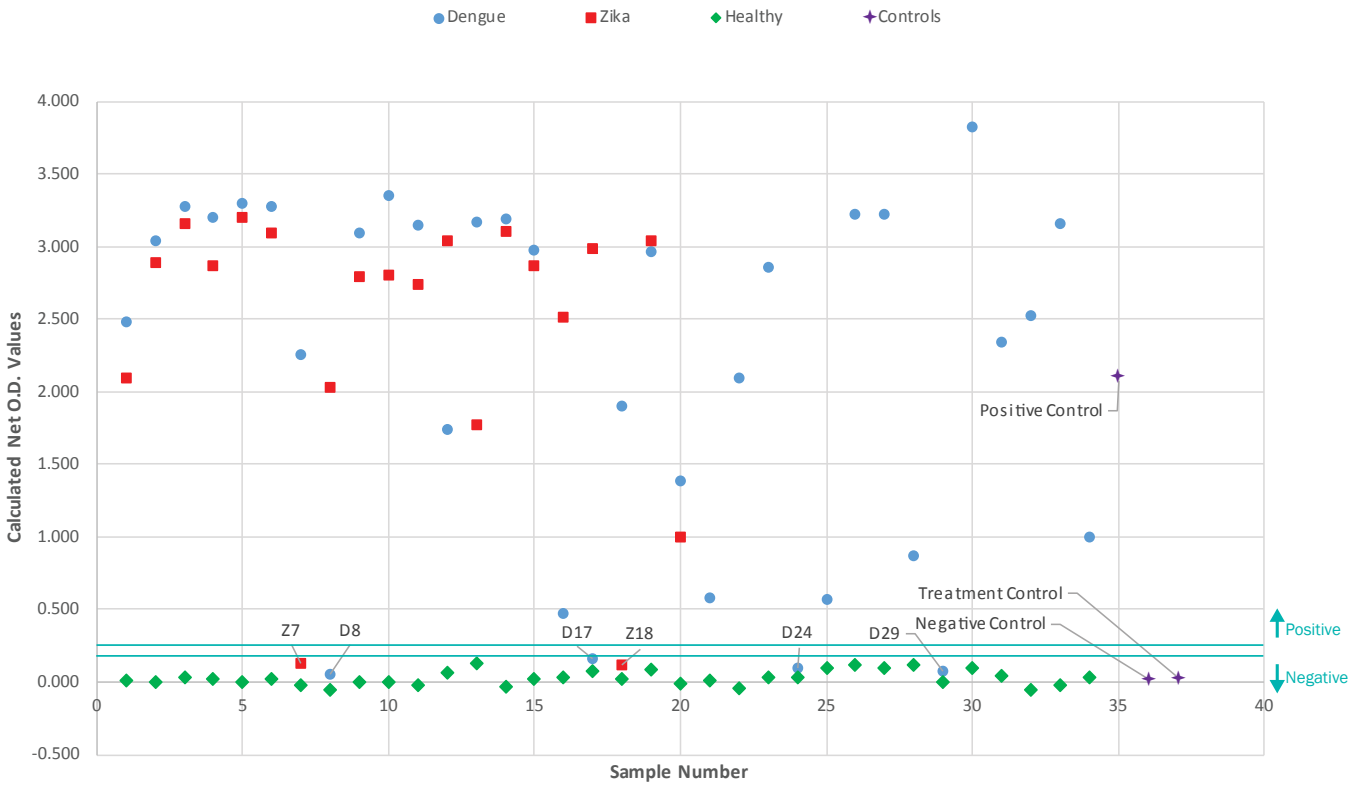


Figure 1: R&D Systems® Anti-Dengue Virus IgG ELISA. Using R&D Systems® Anti-Dengue Virus IgG ELISA, 34 Dengue patient samples (shown in blue) were tested, of which 30 were positive and 4 were negative. 20 Zika patient samples (shown in red) were tested, of which 2 (Z7 and Z18) measured negative and 18 meausred positive for Anti-Dengue Virus IgG. Apparently healthy donor samples are shown in green. See Figure 2 for additional test results further qualifying the 4 Dengue samples negative for Anti-Dengue Virus IgG (D8, D17, D24 and D29) and the 18 Zika samples positive for Anti-Dengue Virus IgG.

Figure 2A - Without Sample Treatment

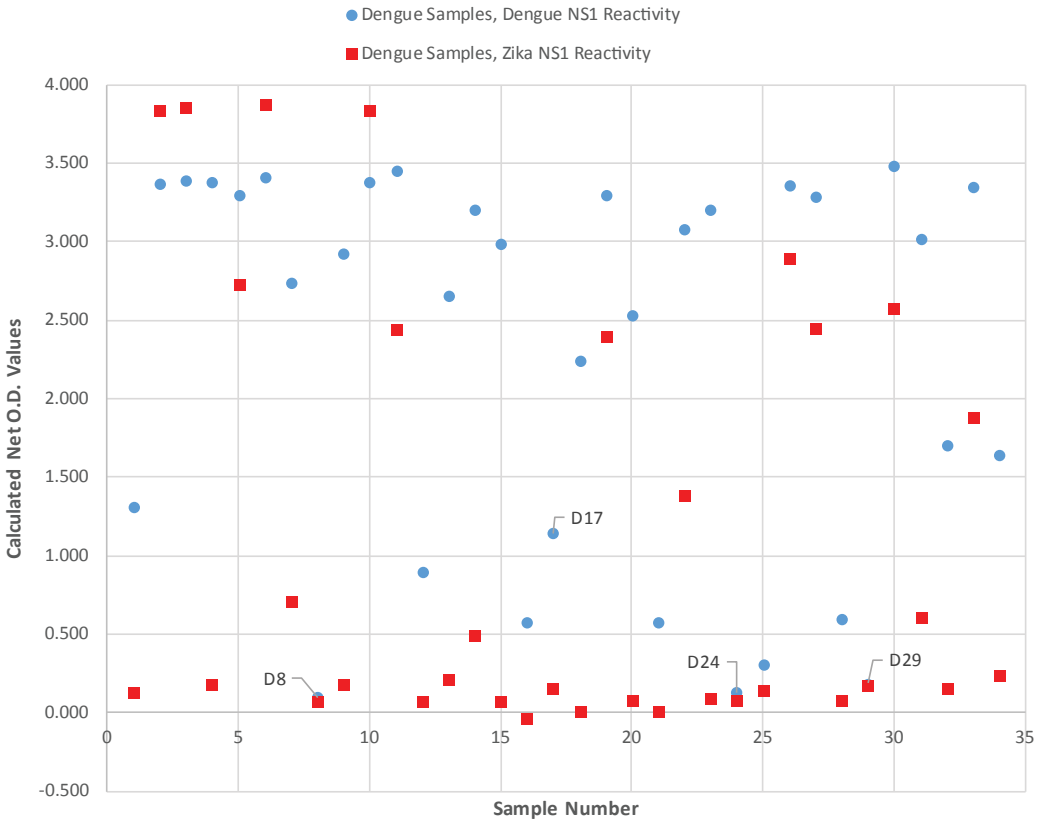


Figure 2B - With Sample Treatment

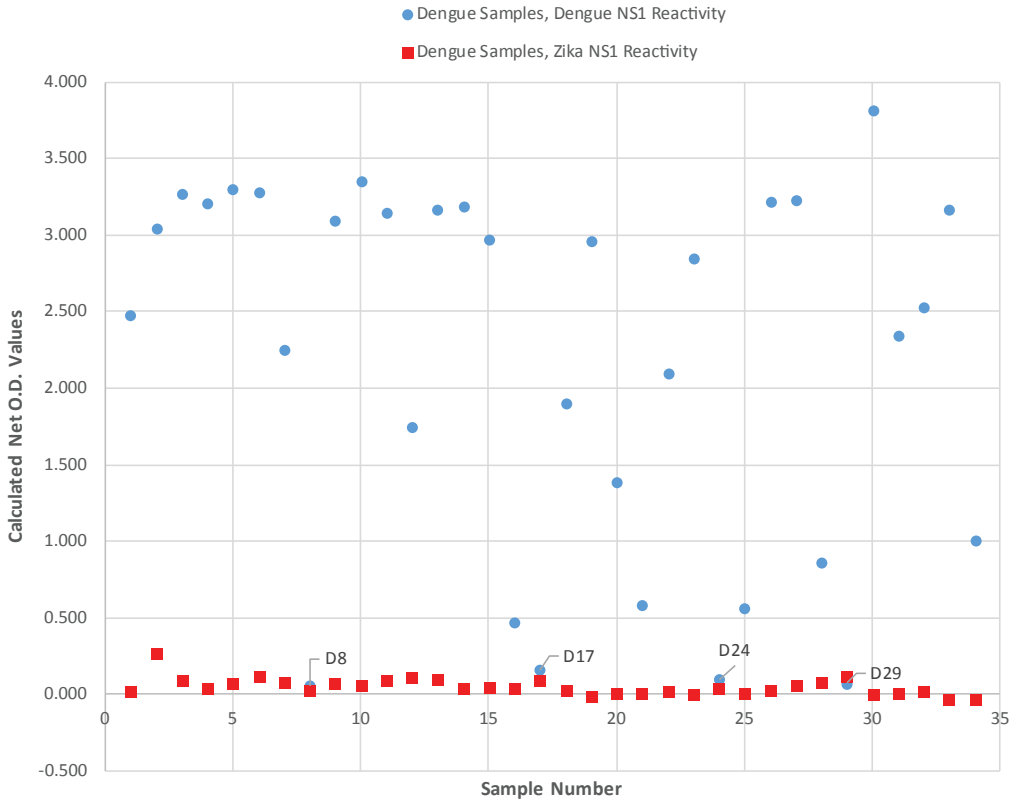


Figure 2C - Without Sample Treatment

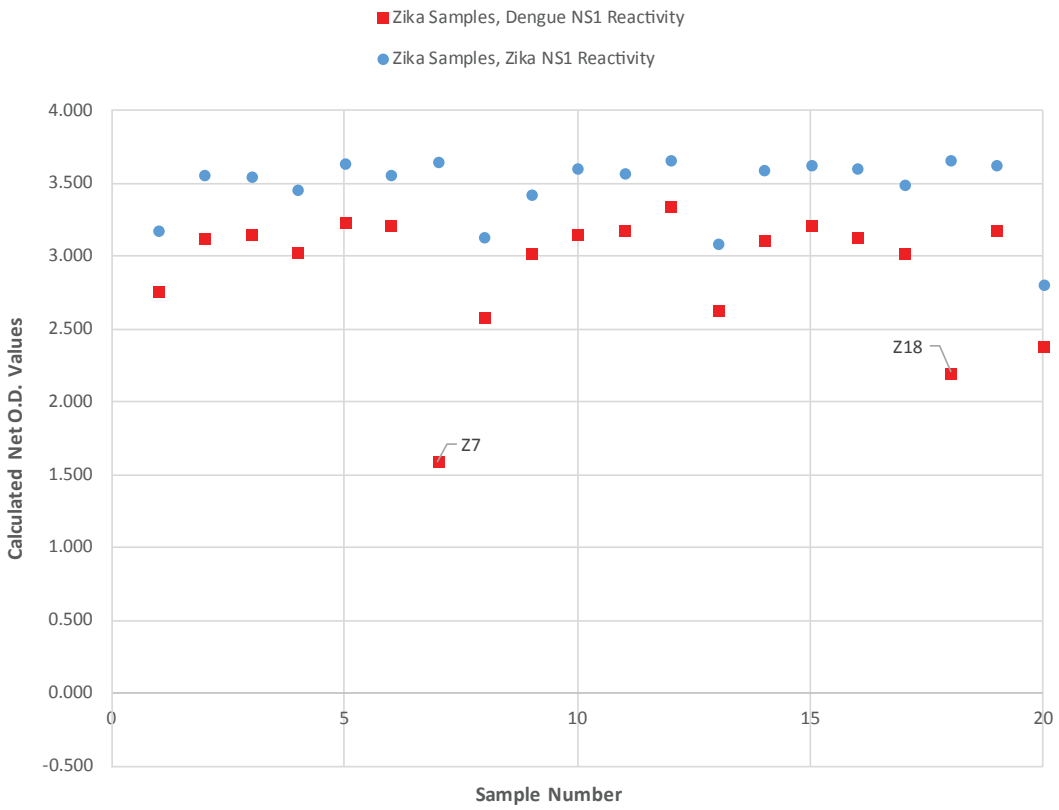
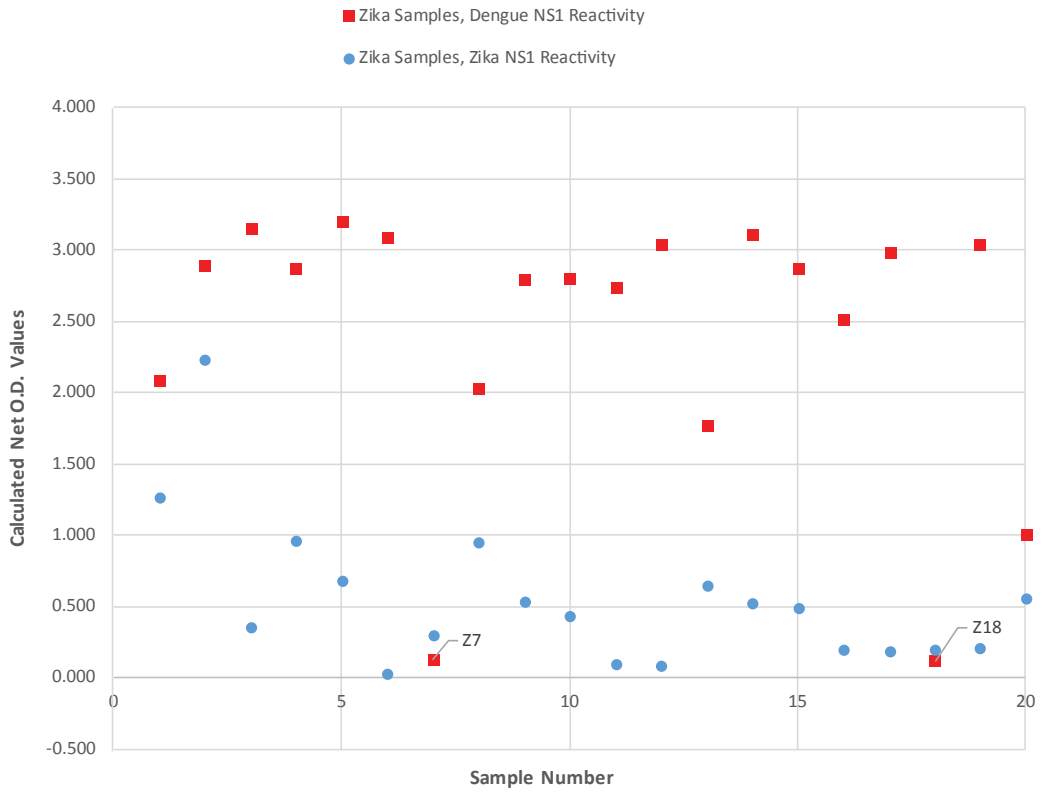


Figure 2D - With Sample Treatment



SAMPLE TESTING DATA *CONTINUED*

Figure 2: Assay results with and without sample treatment. 34 Dengue patient samples (A, B) and 20 Zika patient samples (C, D), either without (A, C) or with (B, D) treatment of the samples with the proprietary treatment reagent included in the Human Anti-Dengue Virus IgG ELISA Kit, were assessed for total IgG antibody reactivity to either Dengue or Zika NS1. Without sample treatment, 15 of the 34 Dengue patient samples had reactivity (net O.D. > 0.4) to Zika NS1 due to cross-reactivity (A), which was minimized following sample treatment (B). Of the 4 Dengue samples that tested negative for Dengue IgG (see Figure 1), the D8, D24, and D29 samples did not have reactive IgG antibodies to Dengue NS1 without sample treatment (A), indicating that these are not Dengue IgG positive samples. The other Dengue IgG negative sample, D17 (see Figure 1), had Dengue NS1 reactive IgG antibodies present without sample treatment (A), but these were no longer reactive following sample treatment (B), indicating that this sample did not contain Dengue specific IgG antibodies, but may contain IgG antibodies to a related flavivirus. For the 20 Zika patient samples (C, D), all had high levels of reactive IgG antibodies to both Dengue and Zika NS1 without sample treatment (C). Following sample treatment, reactivity to Zika NS1 was reduced in all samples, while reactivity to Dengue NS1 remained high in 18 of the 20 Zika samples (D), indicating that these samples are Zika IgG and Dengue IgG double positive. The other 2 Zika samples (Z7 and Z18) had no reactivity to Dengue NS1 following treatment (D), indicating that only these two samples are Zika IgG single positive samples. By using this analysis to compare patient samples before and after treatment to remove flavivirus cross-reactive antibodies, we were able to conclude that some of the samples classified as Dengue IgG positive by the supplier (D8, D24, and D29) were actually Dengue IgG negative, indicating that the supplier's test gave false positive results. Similarly, 18 of the 20 samples classified as Zika IgG positive, also tested positive for Dengue IgG, demonstrating that these were actually Zika IgG and Dengue IgG double positive samples.

PRECISION

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

Inter-Assay Precision (Precision between assays)
Three Anti-Dengue Virus IgG positive samples with low, middle, and high 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.	0.838	1.776	2.811	0.709	1.556	2.756
Standard deviation	0.019	0.033	0.039	0.079	0.151	0.276
CV (%)	2.3	1.9	1.4	11.1	9.7	10.0

REFERENCES

1. Kularatne, S.A. (2015) BMJ, **351**:h4661.
2. Bhatt, S. *et al.* (2013) Nature, **496**:504.
3. Musso, D. *et al.* (2016) Clin. Microbiol. Rev. **29**:487.
4. Cabral-Castro, M.J. *et al.* (2016) J. Clin. Virol. **82**:108.

PLATE LAYOUT

Use this plate layout to record controls and samples assayed.

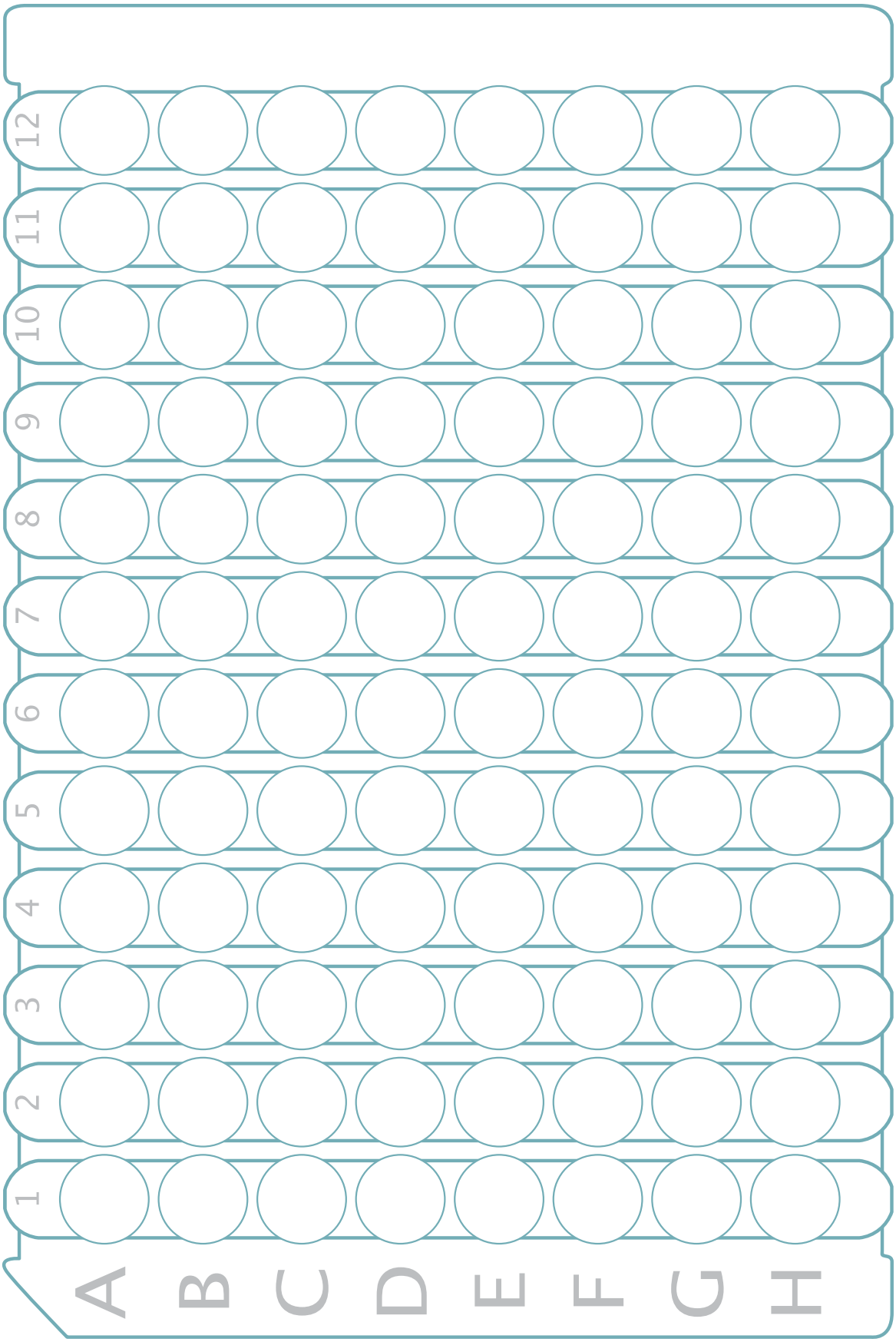


PLATE LAYOUT

Use this plate layout to record controls and samples assayed.

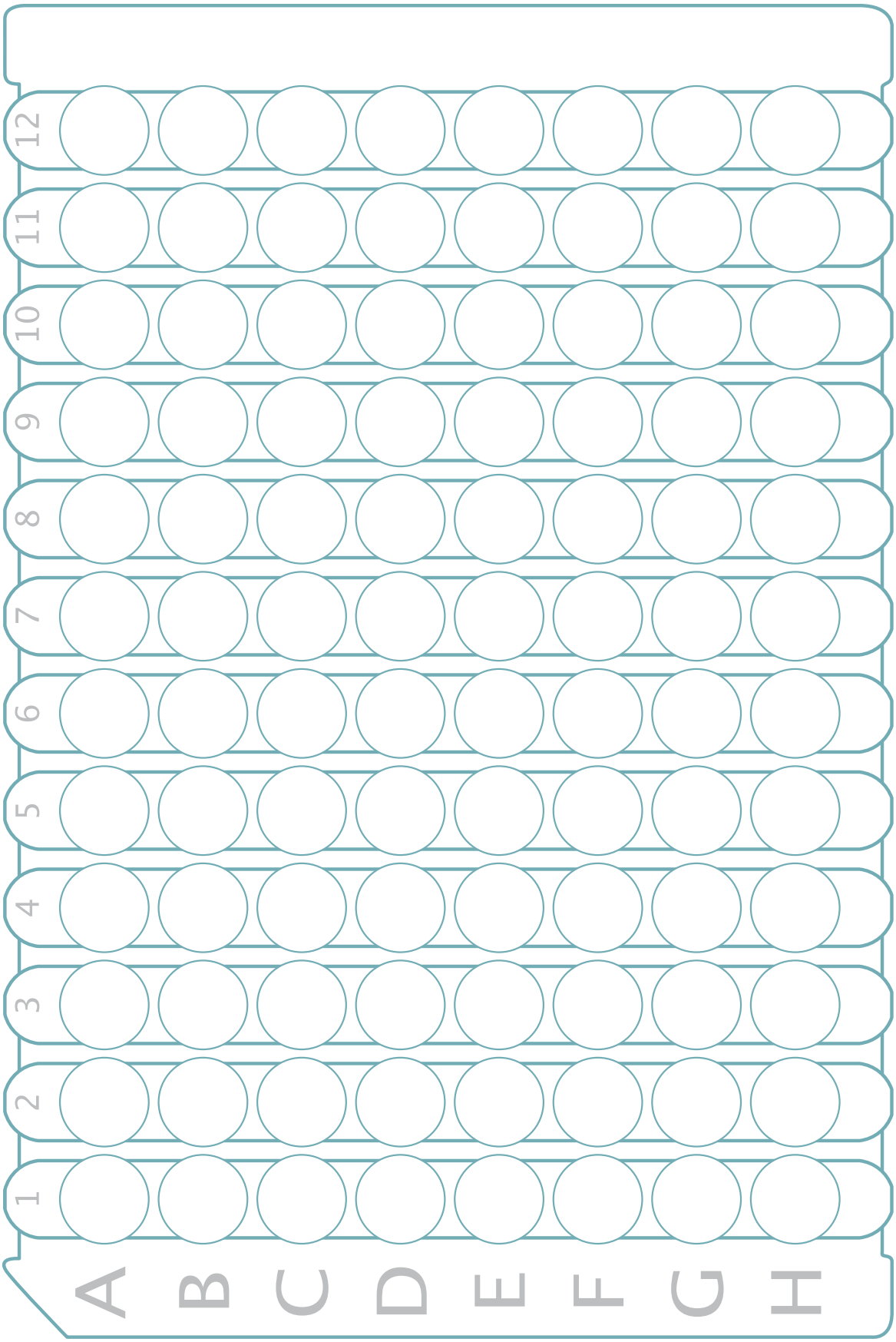
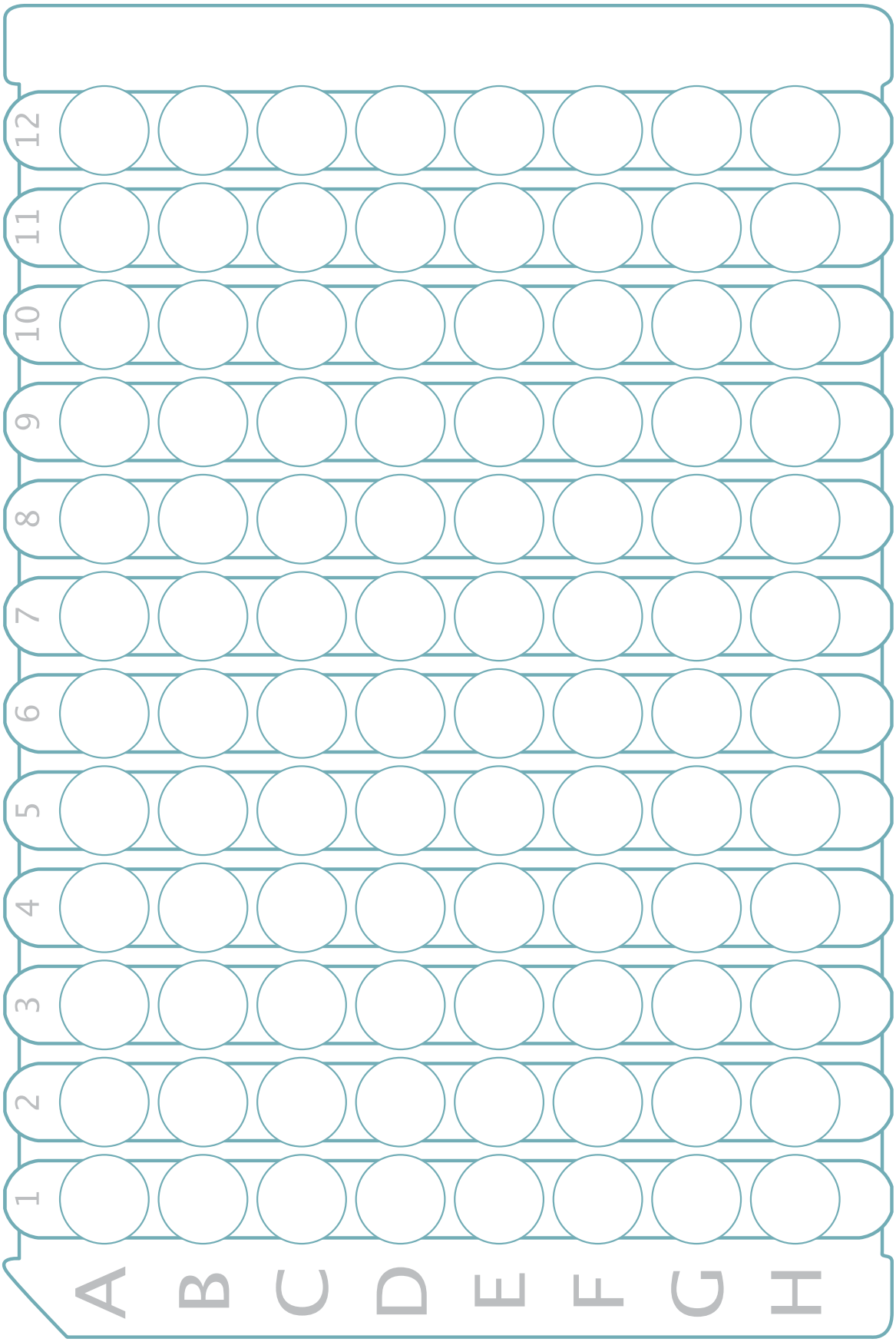


PLATE LAYOUT

Use this plate layout to record controls and samples assayed.



NOTES