



Dengue Virus IgM Elisa

KAPDDENM



DENGUE VIRUS IgM ELISA

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KAPDDENM
IN VITRO DIAGNOSTIC USE

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1. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM antibodies to Dengue Virus subtypes 1,2,3 & 4 in human plasma and sera.

For "in vitro" diagnostic use only.

2. INTRODUCTION

Dengue virus is a member of the virus family *Flaviviridae* and is transmitted to people through the bite of the mosquitoes *Aedes aegypti* and *Aedes albopictus*.

Each year, 100 million people become infected with dengue virus. However, the majority of deaths that result from dengue infection result from Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

The incubation period of dengue fever is approximately four days. It is difficult to distinguish dengue fever from other viral diseases and the person usually recovers in 5 days.

DHF has a similar incubation period as dengue fever and many of the same symptoms. However, the fever is more severe and the drowsiness and lethargy is more extreme. This can cause the individual to lose blood volume, result in hypotension, go into shock (DSS) and die.

It is important to understand why an individual will develop DHF/DSS. The Dengue virus has been shown to have 4 subtypes. These 4 subtypes are different strains of dengue virus that have 60-80% homology between each other. After a person is infected with dengue, they develop an immune response to that dengue subtype. The immune response produced specific antibodies to that subtype's surface proteins that prevents the virus from binding to macrophage cells (the target cell that dengue viruses infect) and gaining entry. However, if another subtype of dengue virus infects the individual, the virus will activate the immune system to attack it as if it was the first subtype. The immune system is tricked because the 4 subtypes have very similar surface antigens. The antibodies bind to the surface proteins but do not inactivate the virus. The immune response attracts numerous macrophages, which the virus proceeds to infect because it has not been inactivated. This situation is referred to as Antibody-Dependent Enhancement (ADE) of a viral infection. This makes the viral infection much more acute. The body releases cytokines that cause the endothelial tissue to become permeable which results in hemorrhagic fever and fluid loss from the blood vessels.

3. PRINCIPLE OF THE TEST

Microplates are coated with highly purified Immunodominant Dengue Virus antigen.

In the 1st incubation, the solid phase is treated with diluted samples and anti Dengue Virus IgM are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti Dengue Virus IgM are detected by the addition of anti hIgM antibody, labeled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Dengue Virus IgM antibodies present in the sample.

Neutralization of IgG anti-DV, carried out directly in the well in the 1st incubation, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

4. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.



Microplate

12 strips x 8 microwells coated with an highly purified Dengue Virus antigen. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.



Negative Control

1x2.0 ml/vial. Ready to use. Pale yellow color coded.

It contains diluted human serum negative for anti Dengue Virus IgM, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.



Positive Control

1x2.0 ml/vial. Ready to use. Green color coded.

It contains diluted human serum positive for anti Dengue Virus IgM, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.



Wash buffer concentrate

1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.



Enzyme Conjugate

1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

CHROM	TMB
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Chromogen/Substrate

1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

STOP	SOLN
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Sulphuric Acid

1x15ml/vial. It contains 0.3 M H₂SO₄ solution.

Attention !: Irritant (Xi R36/38; S2/26/30)

DIL	SPE
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Specimen Diluent

2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

NEUTR	SOLN
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Neutralizing Reagent

1x8ml/vial. It contains goat anti hlgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

2 Plate sealing foils

1 Package insert

5. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10 µl) and disposable plastic tips.
2. ELA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minutes range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

6. WARNINGS AND PRECAUTIONS

For "in vitro" diagnostic use only.

The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

1. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
2. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
3. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
4. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
6. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
7. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
8. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
9. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
10. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
12. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

7. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°-8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

8. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of conservation.

In this case call DIAsource's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°-8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Controls

Ready to use. Mix well before use.

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: Once diluted, the wash solution is stable for 1 week at +2-8° C.

Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent

Ready to use component. Mix carefully on vortex before use.

Neutralizing Reagent

Ready to use. Mix carefully on vortex before use.

Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Legend: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

9. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O "Internal quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

- When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
- DIAsource's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

10. PRE ASSAY CONTROLS AND OPERATIONS

- Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
- Check that the liquid components are not contaminated by visible particles or aggregates.
- Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
- Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- Check that all the other equipment is available and ready to use.
- In case of problems, do not proceed further with the test and advise the supervisor.

11. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

- Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Controls as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- Dispense 50 µl Neutralizing Reagent in all the wells, except A1 used for blanking operations and in the wells used for the Controls.
- Dispense 100 µl of Negative Control in duplicate, 100 µl of Positive Control in duplicate and 100 µl of diluted samples in each properly identified well.
- Incubate the microplate for **60 min at +37°C**.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

- Wash the microplate with an automatic as reported previously (section 9.3).
- Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) in the dark for 20 minutes**.

Important note: Do not expose to strong direct illumination. High background might be generated.

- Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and the positive samples from blue to yellow.
- Measure the colour intensity of the solution in each well, as described in section 9.5, at 450nm filter (reading) and possibly at 620-630nm (background subtraction), blanking the instrument on A1.

General Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

12. ASSAY SCHEME

Method	Operations
Negative & Positive Controls	100 µl
Neutralizing Reagent	50 µl
Samples diluted 1:101	100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4										
B	NC	S5										
C	NC	S6										
D	PC	S7										
E	PC	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Legend: BLK = Blank NC = Negative Control
PC = Positive Control Sn = Samples

13. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control	< 0.150 OD450nm
Positive Control	≥ 0.750 OD450nm
Mean CV%	< 30%

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control > 0.150	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one); 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.750	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

14. RESULTS

If the test turns out to be valid, results are calculated from the mean OD450nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

$$\text{Cut-Off} = \text{NC} + 0.250$$

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to generate the correct interpretation of results.

15. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm value (S) and the cut-off value (Co), or S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not developed IgM antibodies to Dengue Virus.

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of an ongoing Dengue Virus infection and therefore the patient should be treated accordingly.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below.

The following data must not be used instead of real figures obtained by the user.

Negative Control: 0.080 – 0.120 – 0.080 OD450nm

Mean Value: 0.100 OD450nm

Lower than 0.150 – Accepted

Positive Control: 1.000 OD450nm

Higher than 0.75

0 – Accepted

Cut-Off = 0.100+0.250 = 0.350

Sample 1: 0.080 OD450nm

Sample 2: 1.800 OD450nm

Sample 1 S/Co < 1.0 = negative

Sample 2 S/Co > 1.2 = positive

16. PERFORMANCES CHARACTERISTICS

Evaluation of Performances has been conducted on panels of positive and negative samples with reference to a CE marked reference kit.

1. Diagnostic Sensitivity and Specificity:

The Diagnostic **Sensitivity** was calculated on a panel of positive samples derived from people undergoing Dengue Virus infection in the early stage.

A value of > 98% was observed when referring to the reference device.

The Diagnostic **Specificity** was calculated on a panel of samples derived from normal people with no clinical sign of Dengue Virus infection.

A value > 98% was observed.

These findings are summarized in the following table.

Sensitivity	> 98 %
Specificity	> 98 %

2. Reproducibility:

A study conducted on two samples of different anti Dengue Virus IgM reactivity, examined in 16 replicates in three separate runs has shown CV% values ranging 10-20% depending on the OD450nm readings.

The variability shown in the tables did not result in sample misclassification.

16. LIMITATIONS

False positivity has been assessed as less than 2% of the normal population, and was mostly due to very high values of RF in the sample. Frozen samples containing fibrin particles or aggregates may generate false positive results.

17. REFERENCES

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	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoabsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
µT	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
PREC AGENT	Precipitating Agent
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
PIPETTE	Pipette
WASH SOLN	Wash buffer