BioVendor Group

ENG

Instructions for Use:
ALDOSTERONE ELISA

Catalogue number:
RCD030R

For research use only!

BioVendor R&D

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HISTORY OF CHANGES

Previous version	Current version
ENG.002.A	ENG.003.A
The appearance of the entire document, format modifications.	

1. INTENDED USE

For the direct quantitative determination of Aldosterone in human serum, plasma and urine by an enzyme immunoassay.

2. PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate wells. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured with a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of aldosterone in the sample. A set of standards is used to plot a standard curve from which the amount of aldosterone in patient samples and controls can be directly read.

3. CLINICAL APPLICATIONS

Aldosterone is a potent mineralocorticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the reabsorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension.
Measurement of aldosterone levels in serum in conjunction with plasma renin activity levels can be used to differentiate between primary and secondary aldosteronism.

Condition	Serum Aldosterone	Plasma Renin
Primary Aldosteronism	High	Low
Secondary Aldosteronism	High	High

The measurement of aldosterone in concert with selective suppression and stimulation tests can be used to further differentiate primary aldosteronism into two basic types:
– Primary aldosteronism caused by an adenoma of one or both adrenals.
– Primary aldosteronism caused by adrenal hyperplasia.
This differentiation is vital in the treatment and management of the disease. The adrenal adenomas respond well to surgery whereas hyperplastic disease of the adrenals is generally better managed medically. In summary, the precise and accurate measurement of aldosterone by enzyme immunoassay can be an important addition to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

4. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is intended for research use only.
2. Practice good laboratory practices when handling kit reagents. This includes:

– Do not pipette by mouth.
– Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
– Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
– Wash hands thoroughly after performing the test.
– Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A calibrator curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) must be included in every run and the obtained values must fall within the acceptable ranges, as stated in the quality control certificate.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the microplate wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it must not be used.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
15. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

5. LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of aldosterone in human serum, plasma and urine. The kit is not calibrated for the determination of aldosterone in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum or plasma or urine.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only the serum/plasma diluent provided upon request may be used to dilute any high serum or plasma samples
5. Do not use the results of this kit as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6. SAFETY CAUTIONS AND WARNINGS

6.1 Potential Biohazardous Material

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.
All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

6.2 Chemical Hazards

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

7. SPECIMEN COLLECTION AND STORAGE

Serum: Approximately 0.2 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.
Plasma: Approximately 0.2 mL of plasma is required per duplicate determination. Collect 4-5 mL of blood into EDTA plasma tubes. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Urine: 24-hour urine into a specimen collection container. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

8. SPECIMEN PRETREATMENT

Serum and plasma: Serum and plasma are loaded directly to the microplate wells; no specimen pretreatment is necessary.

Urine: Dilute urine samples 1:50 in urine diluent right before the test. Do not stored diluted urine samples.
Example: To 0.98 mL of urine diluent, add 20 µL of the urine sample.

9. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 100, 150 and 350 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).
6. Urine Diluent - Required for diluent of urine specimens before assaying. Available in any quantity.
7. Serum and Plasma Diluent - Required if high samples (>1000 pg/mL) are to be tested again. Available in any quantity.

10. REAGENTS PROVIDED

10.1 Anti-Aldosterone Polyclonal Antibody Coated Microplate Plate-Break Apart Wells

Ready To Use.
Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a re-sealable pouch with desiccant.
Storage: Refrigerate at 2-8ºC
Stability: 12 months or as indicated on label.

10.2 Aldosterone-Horse Radish Peroxidase (HRP) Conjugate

Ready to Use.
Contents: Aldosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 15 mL/bottle
Storage: Refrigerate at 2-8ºC
Stability: 12 months or as indicated on label.

10.3 Aldosterone Calibrators - Ready To Use.

Contents: Six vials containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of aldosterone.
Calibrator concentrations*: 0, 15, 50, 200, 500 and 1000 pg/mL.
* Approximate value - please refer to vial labels for exact concentrations.
Volume: Calibrators A-F: 1 mL/vial
Storage: Refrigerate at 2-8ºC
Stability: 12 months in unopened vials or as indicated on label.

10.4 Controls - Ready To Use.

Contents: Two vials containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of aldosterone.
Refer to vial labels for acceptable ranges.
Volume: 1 mL/vial
Storage: Refrigerate at 2-8ºC
Stability: 12 months in unopened vial or as indicated on label.

10.5 Wash Buffer Concentrate - Requires Preparation. **(10x)**

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2-8ºC
Stability: 12 months or as indicated on label.
Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

10.6 TMB Substrate - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2-8ºC
Stability: 12 months or as indicated on label.

10.7 Stopping Solution - Ready To Use.

Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2-8ºC
Stability: 12 months or as indicated on label.

11. ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	After all kit components have reached room temperature mix gently by inversion. If serum or plasma samples are being used there is no sample preparation required. If urine samples are being used, they must be diluted prior to use (see Specimen Preparation section). Prepare the **working wash buffer** (see wash buffer concentrate under the Reagents Provided section).
2.	Remove the required number of strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3.	Pipette 50 µL of each calibrator, control and specimen sample (serum, plasma or diluted urine) into correspondingly labelled wells in duplicate.
4.	Pipette 100 µL of the aldosterone-HRP conjugate into each well (the use of a multichannel pipette is recommended).
5.	Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 60 minutes at room temperature.
6.	Wash the wells 3 times each time with 350 µL/well of working wash buffer solution. After washing tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
7.	Pipette 150 µL of the TMB substrate into each well at timed intervals (the use of a multichannel pipette is recommended).
8.	Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 20 minutes at room temperature or until calibrator A attains dark blue colour for desired OD.
9.	Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7 (the use of a multichannel pipette is recommended). Mix thoroughly by gently shaking the plate by hand.
10.	Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.

12. CALCULATIONS

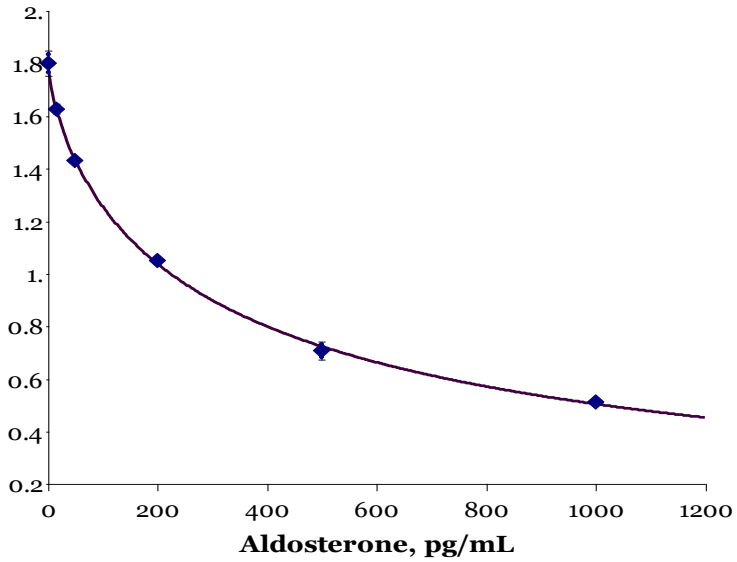
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the serum and plasma samples directly off the calibrator curve.
5. Read the values of the urine samples directly off the curve and multiply by a factor of 50. Next, multiply by the volume of collected 24-hour urine (in mL) to obtain values in pg/24 hour. Finally, divide the pg/24 hour values by $1x10^6$ (1,000,000) to obtain values in µg/24 hour.
6. If a serum or plasma sample reads greater than 1000 pg/mL then dilute it with the Serum and Plasma Diluent (available separately) at a dilution of no more than 1:8. The result obtained must be multiplied by the dilution factor. If a urine sample reads more than 1000 pg/mL then dilute it with the urine diluent at a dilution of no more than 1:8 (from the original 1:50 dilution). The result obtained must be multiplied by the dilution factor.

13. TYPICAL TABULATED DATA

Calibrator	Mean OD (450 nm)	Aldosterone (pg/mL)
A	1.804	0
B	1.63	15
C	1.43	50
D	1.05	200
E	0.71	500
F	0.51	1000
Unknown	1.11	162

14. TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 Sensitivity

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low value sample and it was calculated as follows:
LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.
The Limit of Detection (LoD) was determined to be **9.1 pg/mL**.

15.2 Specificity (Cross reactivity)

The following compounds were tested for cross-reactivity with aldosterone cross-reacting at 100%:

Steroid	%Cross Reactivity
Aldosterone	100
Androsterone	0.01
Cortisol	0.01
Dihydrotestosterone	0.01
11-Deoxycorticosterone	0.075
Testosterone	0.009

The following compounds were tested and cross-reacted at less than 0.001%: Caffeine, Cholesterol Cortisone and DHEAS.

15.3 Interference

The following substances were tested and did not show significant interference in the assay: hemoglobin up to 4 g/L, bilirubin conjugated and free up to 125 mg/L and triglycerides up to 30 mg/mL.

15.4 Intra –Assay precision

Four serum and four urine samples were assayed 24 times each on the same calibrator curve. The results are tabulated below:

Sample	Mean (pg/mL)	SD (pg/mL)	%CV
Serum Sample 1	81.2	7.6	9.4
Serum Sample 2	284.5	25.9	9.1
Serum Sample 3	403.0	22.2	5.5
Serum Sample 4	529.5	36.5	6.9

Sample	Mean (pg/mL)	SD (pg/mL)	%CV
Urine Sample 1	41.2	5.1	12.5
Urine Sample 2	335.8	24.9	7.4
Urine Sample 3	604.7	36.4	6.0
Urine Sample 4	865.8	61.4	7.1

15.5 Inter–Assay precision

Five serum samples were assayed in 20 different tests in the span of at least ten days. The results are tabulated below:

Sample	Mean (pg/mL)	SD (pg/mL)	CV%
1	80.8	10.4	12.8
2	209.0	22.5	10.7
3	454.5	51.9	11.4
4	677.3	79.1	11.7
5	902.3	68.4	7.6

15.5 Recovery

Spiked samples were prepared by adding defined amounts of aldosterone to three patient serum and urine samples. The results are tabulated below:

Sample	Observed Result (pg/mL)	Expected Result (pg/mL)	Recovery %
Serum Sample 1	45.8	-	-
+100 pg/mL	146.5	145.8	100.5
+200 pg/mL	232.5	245.8	94.6
+400 pg/mL	376.1	445.8	84.4
Serum Sample 2	77.8	-	-
+100 pg/mL	203.5	177.8	114.5
+200 pg/mL	302.4	277.8	108.9
+400 pg/mL	444.2	477.8	93.0
Serum Sample 3	82.7	-	-
+100 pg/mL	183.1	182.7	100.2
+200 pg/mL	260.9	282.7	92.3
+400 pg/mL	401.3	482.7	83.1

Sample	Observed Result (pg/mL)	Expected Result (pg/mL)	Recovery %
Urine Sample 1	32.6	-	-
+100 pg/mL	151.9	132.6	114.6
+200 pg/mL	261.0	232.6	112.2
+400 pg/mL	374.6	432.6	86.6
Urine Sample 2	59.7	-	-
+100 pg/mL	185.0	159.7	115.8
+200 pg/mL	297.3	259.7	114.5
+400 pg/mL	436.0	459.7	94.8
Urine Sample 3	73.6	-	-
+100 pg/mL	190.5	173.6	109.7
+200 pg/mL	316.6	273.6	115.7
+400 pg/mL	449.8	473.6	95.0

15.6 Linearity

Patient serum samples were diluted with Serum and Plasma Diluent. Patient urine samples were diluted with Urine Diluent after an initial dilution of 1:10 in Urine Diluent. The results are tabulated below:

Sample	Observed Result (pg/mL)	Expected Result (pg/mL)	Recovery %
Serum Sample 1	241.8	-	-
1:2	127.8	120.9	105.7
1:4	65.0	60.5	107.6
1:8	31.6	30.2	104.5
Serum Sample 2	840.8	-	-
1:2	456.7	420.4	108.6
1:4	217.1	210.2	103.3
1:8	125.0	105.1	118.9
Serum Sample 3	1152	-	-
1:2	624.3	575.9	108.4
1:4	282.3	287.9	98.1
1:8	123.2	144.0	85.6

Sample	Observed Result (pg/mL)	Expected Result (pg/mL)	Recovery %
Urine Sample 1	320.1	-	-
1:2	178.7	160.1	111.7
1:4	92.8	80.0	116.0
1:8	34.1	40.0	85.1
Urine Sample 2	442.3	-	-
1:2	231.9	221.1	104.9
1:4	126.5	110.6	114.9
1:8	48.5	55.3	87.8
Urine Sample 3	572.2	-	-
1:2	290.2	286.1	101.4
1:4	149.4	143.0	104.4
1:8	65.2	71.5	91.1

15.7 Comparative studies

The BioVendor Aldosterone ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 42 serum samples yielded the following linear regression results:
$y = 0.84x + 3.50$, $r = 0.96$

16. REFERENCE VALUES-SERUM / PLASMA

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	n	95% Confidence Range (pg/mL)
Normal Salt Intake, Upright	183	0 – 199

17. REFERENCE VALUES-URINE

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	n	95% Confidence Range (µg/24 hr)
Normal Salt Intake	42	2.8 - 13

18. REFERENCES

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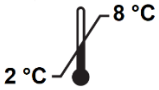
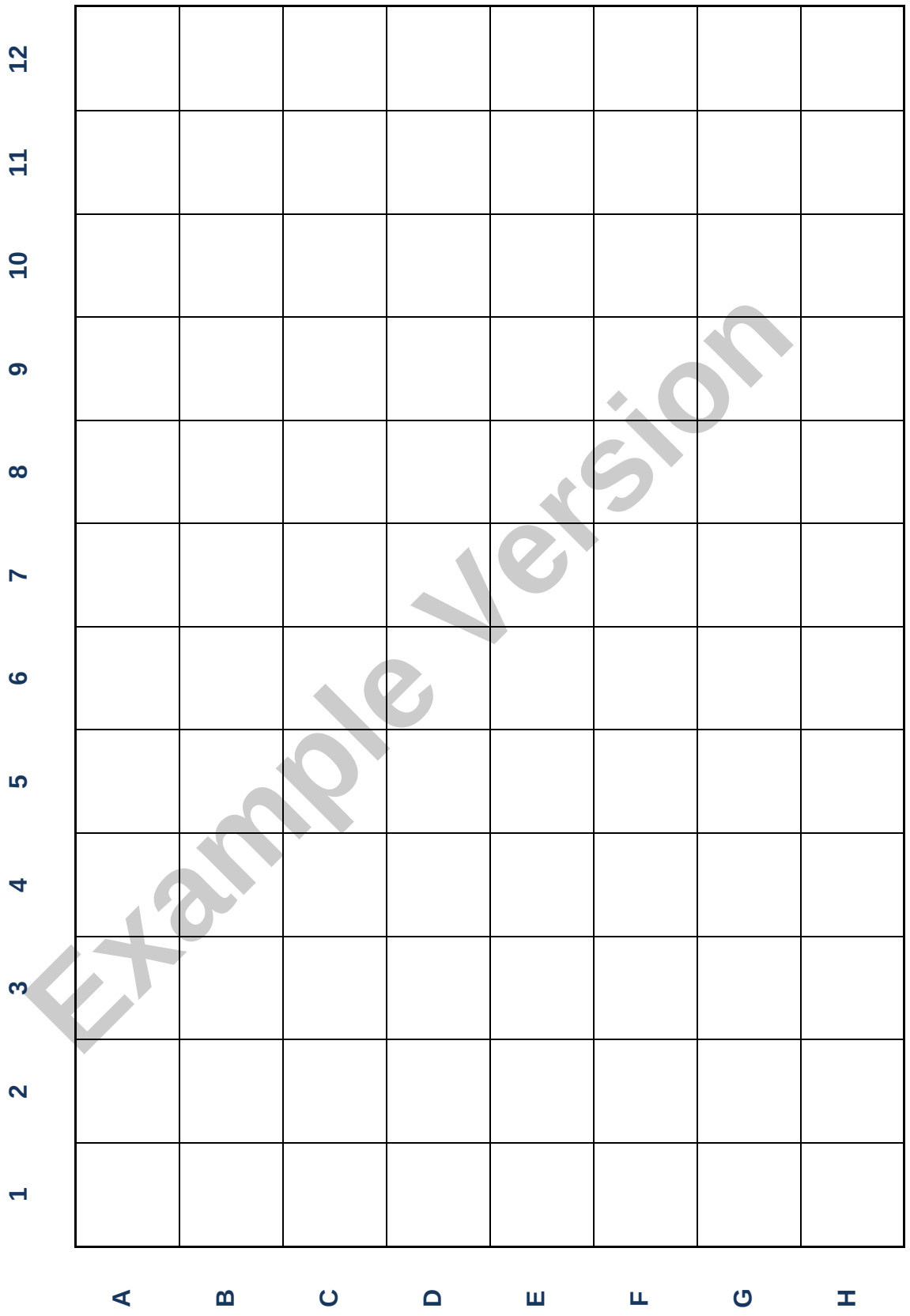
19. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



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