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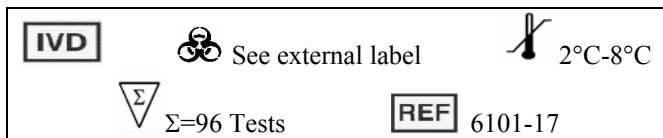
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ISO 13485-2003



Cortisol ELISA

Cat. No. 6101-17

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Cortisol concentration in serum and plasma.

PRINCIPLE OF THE ASSAY

Cortisol (antigen) in the sample competes with horseradish peroxidase-Cortisol (enzyme-labeled antigen) for binding onto the limited number of anti-Cortisol (antibody) sites on the microplates (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme substrate (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined.

Cortisol concentration in the sample is calculated based on a series by a set of standard.

The color intensity is inversely proportional to the Cortisol concentration in the sample.

COMPONENTS



96 Tests

- **MICROWELL PLATE** 1plate

Blank microwell strips fixed on a white strip holder. The plate is sealed in a pouch with desiccant.

8x12/12x8-well strips per plate. Anti-Cortisol-IgG adsorbed on microplate.

The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.

- **Cortisol Standards*** 5vials

STD₀ (1 vial) 1 mL

STD₁ (1 vial) 1 mL

STD₂ (1 vial) 1 mL

STD₃ (1 vial) 1 mL

STD₄ (1 vial) 1 mL

*. Standard (S₀,S₁,S₂,S₃,S₄) (liquid)

The standard has the following concentration of Cortisol:

	S ₀	S ₁	S ₂	S ₃	S ₄
ng/ml	0	10	50	150	500

Stability: until the expiration date printed on the kit.

When the vial is open the standard is stable six months at +4°C.

- **HRP-CONJUGATE REAGENT** 1bottle
21 ml per vial.
Horseradish peroxidase-conjugated Cortisol.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
- **TMB SUBSTRATE** 1bottle
12 ml per vial.
 H_2O_2 -TMB 0.25gr/L (avoid any skin contact)
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
- **STOP SOLUTION** 1bottle
12 ml per bottle.
sulfuric acid solution (0.15M H_2SO_4).
Ready to use as supplied.
- **PLASTIC SEALABLE BAG** 1unit
For enclosing the strips not in use.
- **PACKAGE INSERTS** 1copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, $37\pm 0.5^\circ C$.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored

between 2-8 °C; **do not freeze**. To assure maximum performance of this Cortisol ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** IVD

FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (0.15M H₂SO₄) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.
19. The enzymatic activity of the HRP-conjugate might be

affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.

20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

ASSAY PROCEDURE

Step1 Reagents preparation: Allow the reagents and samples to reach room temperature (**18-30°C**) for at least 15-30minutes.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells. As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S₀-S₄), two for each sample and one for Blank.

Step3 Adding Sample and HRP-Conjugate
Pipette:

	Standard	Sample	Blank
Sample	---	20 µl	---
Standards S ₀ -S ₄	20 µl	---	---
Conjugate	200 µl	200 µl	---

Step4 Incubating: Incubate for **60minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step5 Washing: At the end of the incubation, Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining liquids.

Step6 Coloring: Pipette

	Standard	Sample	Blank
TMB-Substrate	100µl	100µl	100µl

Incubate the plate at **37°C for 15minutes, avoiding light**.

Step7 Stopping Reaction: Pipette: Stop Solution into each well and mix gently.

	Standard	Sample	Blank
Stop solution	100µl	100µl	100µl

Step8 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**.

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

STANDARD CURVE - CALCULATION OF RESULTS

Mean absorbance and relative percentage

Calculate the mean of the absorbances (E_m) for each point of the standard curve and of each sample. Express data as the percentage of the mean absorbance of B₀ (E_mB₀) with the following formula:

$$(B/B_0)\% = \frac{E_m}{(E_m S_0)} \times 100$$

Standard curve

Plot the values of the standards expressed as (B/B₀)% on the enclosed logit-log paper.
Extrapolate the line passing through the points.

Calculation of results

Interpolate the values of the samples expressed as (B/B₀)% on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

REFERENCE VALUES

The serum or plasma Cortisol reference value are:

60 - 230 ng/mL

Patient treated with ACTH; 280 - 600 ng/mL

Patient treated with dexamethasone 0 - 50 ng/mL

TEST PERFORMANCE AND EXPECTED RESULTS

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Cortisol	100.0	%
Cortisone	10.8	%
11 α deoxicortisol	18.7	%
corticosterone	2.4	%
Progesterone	0.1	%
aldosterone	0.01	%
11α OH-Progesterone	0.01	%
Cholesterol	<1x10 ⁻⁶	%

Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B₀, is 1 ng/mL when the value of (B/B₀)% is approx 90%.

Precision

The inter and intra-run precision had a coefficient of variation of 3.2% and 5.8% respectively.

Accuracy

The recovery of 10 - 50 - 150 - 500 ng/mL of Cortisol added to "plasma-free" sample gave an average value (±SE) of 105% ± 4.8% with reference to the original concentrations.

Correlation with RIA

Correlation with RIA performed on the same samples:

$$y = 5.71 + 1.08 x$$
$$r = 0.985$$
$$n = 25$$
$$p < 0.001$$

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of the ELISA method. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antibodies may be also undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment,

timing, volumes, sample nature and quality.

4. The prevalence of the marker will affect the assay's predictive values.
5. This is a qualitative assay and the results cannot be used to measure antibodies concentrations.
6. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

1. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones

VALIDITY

Please do not use this kit beyond the expiration indicated on the kit box and reagent labels

REFERENCES:

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