Urinary Cortisol ELISA Kit

Cat.No:DEIA1729

Lot. No. (See product label)

PRODUCT INFOMATION

Storage	1. Store all reagents at 2 °C – 8 °C in the dark. 2. Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use. 3. Do not remove the adhesive sheet from the unused strips.
Pkg#Size	96T
Intended use	Competitive immunoenzymatic colorimetric method for determination of Free Cortisol concentration in Urine.
Principle Of The Test	Urinary Cortisol (antigen) in the sample competes with horseradish-peroxidase Cortisol (enzyme-labelled-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 (H2O2) and the TMB-Sustrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined. Urinary Cortisol concentration in the sample is calculated based on a series of standard. The colour intensity is inversely proportional to the urinary cortisol concentration in the sample.
Reagents And Materials Provided	1. Cortisol Standards STD0 – STD4, 5 vials, 1 mL each 2. Incubation buffer, 1 vial, 100 mL Phosphate buffer 50 mM pH 7.4; BSA 1 g/L 3. Conjugate, 1 vial, 1.0 mL Cortisol-HRP conjugate 4. Coated Microplate, 1 breakable microplate Anti-Cortisol-IgG adsorbed on microplate 5. TMB-substrate, 1 vial, 15 mL H2O2-TMB 0.26 g/L (avoid any skin contact) 6. Stop solution, 1 vial, 15 mL Sulphuric acid 0.15 mol/L (avoid any skin contact) 7. Low Control, 1 vial, 1 mL Ready to use 8. High Control, 1 vial, 1 mL Ready to use
Materials Required But Not Supplied	1. Distilled water 2. Automatic dispenser 3. Microplates reader (450 nm)
Reagent Preparation	Preparation of the Standard (S0, S1, S2, S3, S4) The standards have the following concentration of Cortisol: Stable when stored at ±4 °C until the expiration date of the kit; once opened the standards are stable six months at 2 °C – 8 °C. Preparation of diluted Conjugate Prepare immediately before use. Add 10 µL Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently for at least 10 minutes with a rotating mixer. Stable for 3 hours at room temperature (22 °C – 28 °C).

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Assay Steps	As it is necessary to perform the determination in duplicate, prepare two wells for each of the four points of the standard curve (S0-S4), one for Blank. 1. Add 10 µL Standard S0-S4 Controls and Diluted samples to their respective wells. 2. Add 300 µL Diluted Conjugate to each well except for the blank. 3. Incubate at 37°C for 1 hour. Remove the contents from each well; wash the wells 2 times with 400 µL of distilled water. 4. Add 100 µL TMB substrate to each well. 5. Incubate at room temperature (22 °C – 28 °C) for 15 minutes in the dark 6. Add 100 µL Stop solution to each well. 7. Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.
Quality Control	Each laboratory should assay controls at normal, high and low levels range of Urinary Cortisol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
Precautions	1. The reagents contain Proclin 300 as preservative. 2. Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants. 3. Maximum precision is required for reconstitution and dispensation of the reagents. 4. Do not use different lots of reagents. 5. Do not use heavily haemolized samples. 6. This method allows the determination of Cortisol from10 ng/mL to 500 ng/mL. 7. The clinical significance of the Cortisol determination can be invalidated if the sample was treated with corticosteroids or natural or synthetic steroids.
Specimen Collection And Handling	The total volume of urine excreted during a 24 hours should be collected and mixed in a single container. Urine samples which are not to be assayed immediately should be stored at 2 °C – 8 °C or at – 20°C for periods longer than a week. Dilute the urine sample (1 ± 1) with Incubation buffer (e.g. 100 µL ± 100 µL)
Calculation	Mean Absorbance Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample Standard Curve Plot the values of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points Calculation of Results Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL. To calculate the cortisol concentration in urine, calculate as above and correct for total volume of volume of urine collected in 24 hours: ng/mL x Vol(mL) urine 24 h/ 1.000 = µg Cortisol/24h
Reference Values	50 – 190 µg/24 hours
General Description	Cortisol, also known more formally as hydrocortisone (INN, USAN, BAN), is a steroid hormone, more specifically a glucocorticoid, produced by the zona fasciculata of the adrenal gland. It is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to increase blood sugar through gluconeogenesis; suppress the immune system; and aid in fat, protein and carbohydrate metabolism. It also decreases bone formation. Various synthetic forms of cortisol are used to treat a variety of diseases.
Interpretation of Results	If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

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Limitations	Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

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