EIASON® Cortisol

IVD

Enzymeimmunoassay for the quantitative determination of cortisol
in human serum or plasma

Kit instruction

For in-vitro use only

Product of

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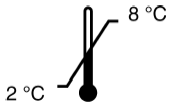
REF E03-004-96

2 °C 8 °C

CE

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	SUB	Substrate
REF	Order number	CAL 0-6	Calibrators
	Product of	STOP	Stop solution
	Storage	WASH	Wash buffer
CE	European Conformity	LOT	Batch code
	Expiry date	MPL	Microplate
CONJ	Conjugate		

Intended use

For in-vitro use only.

The EIASON® Cortisol ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Cortisol in serum and plasma.

Summary

Cortisol /hydrocortisone, compound F) is the main corticosteroid secreted in humans by the adrenal cortex. This steroid hormone has a molecular weight of 363.5. In most physiological conditions, only about 10% of plasma cortisol circulates unbound from transcortin and albumin. Among the products of the human adrenal cortex, only cortisol is involved in the regulation of adrenocorticotropic hormone (ACTH) secretion. As the level of free (non-protein bound) cortisol in blood rises, the release of ACTH is inhibited by the negative feedback effect.
Conversely, if cortisol levels are subnormal, the negative feedback decreases, ACTH levels rise, and the adrenal cortex secretes cortisol until normal blood levels are restored. The release of ACTH is under control of hypothalamic corticotrophin-releasing hormone (CRH); the negative feedback system involving cortisol has been identified at both hypothalamic and pituitary levels. (1). Normally during the day there is a fluctuation of cortisol achieving the highest level in the morning and the lowest in the night. Useful information is given when cortisol measurement is done in samples withdrawn at a fixed hour (8.00 a.m.). The main biological effects of cortisol are: promotion of gluconeogenesis, deposition of liver glycogen, increase in blood glucose concentration when the carbohydrate utilization is reduced, effect on fat metabolism and anti-inflammatory action. Cortisol measurement is a powerful tool for the evaluation of suspected abnormalities in glucocorticoid production: Cushing's syndrome (hypercortisolism), Addison's disease or secondary adrenal insufficiency (hypocortisolism). In many cases, it is necessary to perform dynamic tests (suppression or stimulation) in order to localize the defect at one of the three main levels (i.e. adrenal, pituitary and hypothalamus).

Assay principle

The EIASON® Cortisol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a monoclonal antibody directed towards an antigenic site on the Cortisol molecule. Endogenous Cortisol of a patient sample competes with a Cortisol-horseradish peroxidise conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Cortisol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Cortisol in the patient sample

Warnings and precautions

The EIASON® Cortisol ELISA Kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is

no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Storage and stability

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Diluted WASH solution is stable for 2 weeks at room temperature.

Specimen collection and storage

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Please note: Samples containing sodium azide should not be used in the assay.

Do not use lipaemic or grossly haemolytic or icteric specimens.

Serum:

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection. (EDTA, Citrate, Heparin).

Storage:

Store specimen for up to 5 days at 2°-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Freeze only once. When required, thaw test sera at room temperature and mix gently to ensure homogeneity.

Specimen dilution:

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with CAL 0 and reassayed as described in the assay procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µl Serum + 90 µl CAL 0 (mix thoroughly)
b) Dilution 1:100: 10 µl dilution a) 1:10 + 90 µl CAL 0 (mix thoroughly).

Materials provided

Allow all reagents 1-6 to reach room temperature before use.

1. MPL ELISA strip wells coated with an anti-cortisol antibody. (96 wells in total, 8 wells per strip). Before opening the packet of strip wells, allow it to stand at room temperature (20-25°C) for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided.

2. CAL 0-6 Calibrators (1 mL each); ready to use:
   Concentrations: 0; 20; 50; 100; 200; 400, 800 ng/mL.
   Conversion: 1 ng/mL = 2.76 nmol/l

3. WASH Wash solution (30 mL, 40 x concentrated);
   dilute to 1.2 l with distilled water before use.
   Diluted WASH solution is stable for 2 weeks at room temperature

4. CONJ Enzyme conjugate 1 vial, 25 mL, ready to use
   Cortisol conjugated to horseradish peroxidase

5. SUB Peroxidase substrate (tetramethyl benzidine; TMB; 14 mL ready to use).

6. STOP Stop solution (0.15 M sulphuric acid; 14 mL ready to use).

Materials required but not provided in the kit

- Pipettes capable of dispensing 20µL, 100 und 200µL
- Distilled water
- ELISA plate reader suitable for 96 well formats and capable of measuring absorbances at 450 nm.

Assay procedure

General remarks

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption. As a general rule the enzymatic reaction is linearly proportional to time and temperature. Calculate the number of individual MPL wells needed for the assay. Allow all the reagents supplied including the appropriate number of strips to reach room temperature, fit the number of strip wells required firmLy into the frame provided.

Each run must include controls and a standard curve.

Assay procedure

1. Pipette 20 µL of CAL 0-6, controls and test sera into the wells (in duplicate).
2. Pipette carefully 200 µL of CONJ into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step
3. Incubate for 1 hour 20°-25°C.
4. After the incubation with CONJ, discard the CONJ by briskly inverting the wells over a suitable receptacle, wash 3 times with 400µL diluted WASH per well and each time tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of wash buffer.
5. Pipette carefully 100 µL of SUB into each well.
6. Incubate for 15 minutes at 20-25°C in the dark during which time a blue colour will develop.
7. Stop the substrate reaction by careful addition of 100 µL of STOP to each well (this will cause the blue colour to turn yellow) and shake the plate for about 5 seconds on a plate shaker to ensure uniformity of the solution in each well. It is most important to ensure that the substrate incubation time (i.e. time from addition of SUB to addition of STOP) is the same for each well.
8. Measure the absorbance of each well at 450 nm as reading filter (and 405 nm as overrange filter if possible) (reference 620 – 650 nm) within 10 minutes after adding the stop solution.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Quality control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance. Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit.
The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IASON directly.

Calculation of results

- Draw a standard curve by plotting the absorbance of each standard against its concentration. Read off the values of the test samples. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.
- Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL (4 parameter logistics) is recommended.

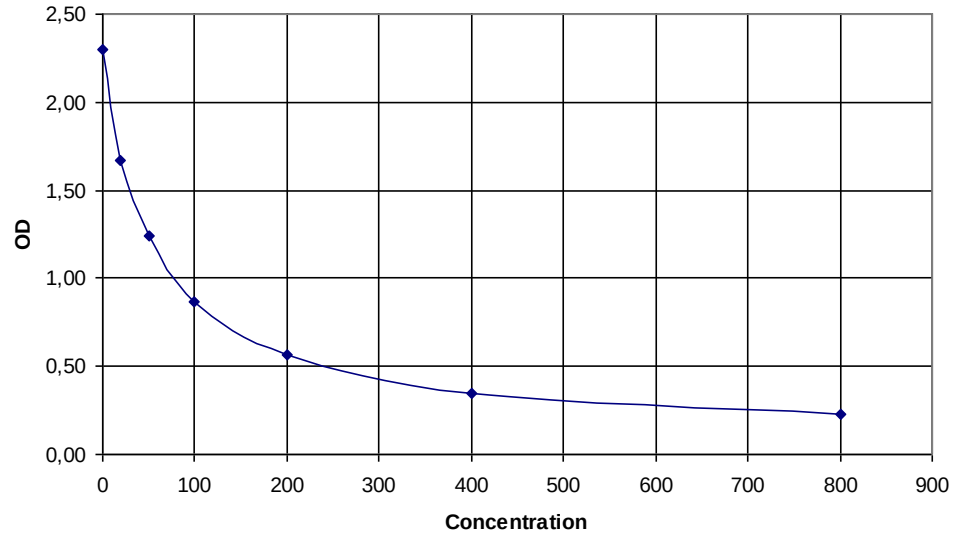
Sample assay data

Example of typical results obtained with EIASON® Cortisol calibrators:

Calibrator [ng/mL]	Absorbance at 450 nm
0	2.30
20	1.67
50	1.24
100	0.87
200	0.57
400	0.35
800	0.23

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



This sample calibration curve is for illustration only.

Expected Values

		ng/mL
between	8:00 10:00 am	50 - 230
	4:00 pm	30 - 150

These values are from Tietz's textbook and may be used as a guideline.

Each laboratory is strongly recommended to determine ranges for their local population. The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Assay characteristics

Assay dynamic range

The range of the assay is between 0 – 800 ng/mL.

Assay specificity

Steroid	Cross-reactivity %
Cortisol	100
Corticosterone	45
Progesteron	9
Deoxycortisol	<2
Dexamethazone	<2
Estrone	<0.01
Estriol	<0.01
Testosterone	<0.01

Analytical sensitivity: **2.5 ng/mL**

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of CAL 0

Precision

Intra-assay-precision (n = 20)			Inter-assay-precision (n = 10)		
Sample	**Concentration (ng/mL)**	**CV [%]**	**Sample**	**Concentration (ng/mL)**	**CV [%]**
A	43.5	8.1	A	55	6.6
B	226.5	3.2	B	209	7.7
C	403.6	5.6	C	361	6.5

Recovery

Samples have been spiked by adding Cortisol solutions with known concentrations in a 1:1 ratio. The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added concentration 1:1 (v/v) [ng/mL]	Measured conc. [ng/mL]	Expected conc. [ng/mL]	Recovery [%]
A	0	57	57	100
	200	110	128.5	86
	400	216	228.5	95
	800	436	428.5	102
B	0	240	240	100
	200	210	220	95
	400	356	320	111
	800	514	520	99
C	0	378	378	100
	200	263	289	91
	400	355	389	91
	800	558	589	95

Linearity

Sample	Dilution	Measured conc. [ng/mL]	Expected conc. [ng/mL]	Recovery [%]
A	None	48.0	48	
	1:2	22.0	22	92
	1:4	12.9	12	108
	1:8	6.0	6.0	100
	1:16	3.3	3.0	110
B	None	255.0	255	
	1:2	118.0	127.5	93
	1:4	63.1	63.8	99
	1:8	34.2	31.9	107
	1:16	15.9	15.9	100
C	None	427	427	
	1:2	190	213.5	89
	1:4	97	106.8	91
	1:8	50	53.4	94
	1:16	25	26.7	94

Interferences

Any improper handling of samples or modification of this test might influence the results.
Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Drug interferences:

Until today no substances (drugs) are known to us, which have an influence to the measurement of Cortisol in a sample.

No high-dose-hook-effect was observed in this test.

LEGAL ASPECTS

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results only even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

USEFUL PUBLICATIONS

1. L. Thomas, Labor und Diagnose, 4. Auflage, 1992
2. Tietz, N.W., Textbook of Clinical Chemistry, Saunders, 1968

Pipetting scheme

Allow all reagents to reach room temperature before use.

1. Pipetting	CAL 0-5 **20 µL**	Controls **20 µL**	Samples **20µL**
2. Pipetting	CONJ **200µL** Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step		
3. Incubation	1 hours at 20° - 25°CC		
4. Washing	wash 3 x : aspirate or decant add 400 µL diluted WASH aspirate or decant and dry on an absorbent material		
5. Pipetting	SUB **100µL**		
6. Incubation	15 min at room temperature (20-25°C)		
7. Pipetting	STOP **100µL**		
8. Reading	450nm (RF 620 – 650nm) Optional Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 10 min. Calculation: 4-parameter or point to point		

Expected Values

		ng/mL
between	8:00-10:00 am	50 - 230
	4:00 pm	30 - 150

These values are from Tietz's textbook and may be used as a guideline.
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