

Corticosterone HS(High Sensitivity) EIA

Enzymeimmunoassay for the
quantitative determination of
corticosterone in serum or plasma

*For Research Use Only. Not for use in diagnostic
procedures.*

Intended Use

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The Immunodiagnostic Systems Limited Corticosterone (High Sensitivity) EIA kit is a competitive enzymeimmunoassay (EIA) for the quantitative determination of corticosterone in serum or plasma. The method incorporates a sample heat treatment step to inactivate binding proteins.

Summary and Explanation

Corticosterone (4-pregnen-11, 21-diol-3, 20-dione) is secreted by the adrenal cortices and is the major glucocorticoid found in birds, reptiles, amphibians, rats and mice, as well as other species. The majority of corticosterone in the circulation is carried by corticosteroid binding globulin. The level of corticosterone can be used as an index of adrenal function. Response can be observed from ACTH release (causing secretion) and by dexamethasone or hypophysectomy (causing suppression). Stress also plays a very important part in the circulating corticosterone levels causing rapid increases under stressful situations including temperature change, experimental stimuli, or unusual routine.

Method Description

The Immunodiagnostic Systems Limited Corticosterone (High Sensitivity) EIA kit is a competitive enzymeimmunoassay utilising a polyclonal corticosterone antibody coated onto the inner surface of polystyrene microtitre wells. Heat treated Calibrators, Controls and samples are incubated with enzyme (horseradish peroxidase) labelled corticosterone in the antibody coated wells for 4 hours at room temperature. The wells are washed and colour is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microplate plate reader, colour intensity developed being inversely proportional to the concentration of corticosterone.

Warnings and Precautions

The Corticosterone (High Sensitivity) EIA kit is for research 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. Immunodiagnostic Systems Limited Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

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.

Preparation of Reagents

Calibrator **CAL** : Calibrator **CAL** is supplied lyophilised. Reconstitute with 1 mL of distilled or deionised water, replace stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Store at 2-8°C.

Controls **CTRL** : Controls **CTRL** are supplied lyophilised. Reconstitute with 0.5 mL of distilled or deionised water, replace stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Store at 2-8°C.

Wash Solution: Add the contents of each bottle of Wash Concentrate **WASHBUF 20x** to 950 mL of distilled or de-ionised water and mix. Store at room temperature.

All other reagents are supplied ready for use.

Allow all reagents to come to room temperature before use. Reagents should be mixed by repeated inversion before use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Reconstituted Calibrator **CAL** and Controls **CTRL** can be stored at 2-8°C for up to 8 weeks.

Unused Antibody Coated Plate strips must be returned to the foil pouch with the desiccant sachet and sealed. Store at 2-8°C for up to 8 weeks.

Wash Solution can be stored at room temperature for up to 8 weeks.

Note:

The specimens' storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on appropriate practices, please refer to the CLSI GP44-A4, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline - Fourth Edition

Indications of possible deterioration of kit reagents

The presence of abnormal particulate matter in any of the reagents.

A decrease in the absorbance of the zero calibrator.

A shift in the slope of the curve from its normal position

Specimen Collection, Storage and Preparation

The assay should be performed using serum or plasma (EDTA or heparin) specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C. Avoid repeated freeze/thaw of samples. Samples that contain more than 15ng/mL corticosterone need to be diluted prior to heat treatment using calibrator diluent. Mouse and rat samples should be diluted 1:20 or 1:40.

The following notes on other types of sample are provided for guidance only. It is the responsibility of the researcher to validate their chosen sample type.

Cell cultures - Cell culture media should be collected and stored at -20°C. Samples that contain more than 15ng/mL corticosterone need to be diluted prior to heat treatment using calibrator diluent.

Tissue samples - Post-hypophysectomy/post-adrenalectomy samples should be frozen immediately after removal and stored at -80°C until required. Prior to analysis, glands should be homogenized or sonicated in appropriate buffer. Buffers described in the literature include Krebs-Ringer bicarbonate buffer (KRB) containing glucose. Samples should be extracted prior to analysis. Samples that contain more than 15ng/mL corticosterone need to be diluted prior to heat treatment using calibrator diluent.

Optional extraction procedure:

(e.g. for post-hypophysectomy, post-adrenalectomy samples)

Step 1: Pipette 10, 25 or 50µL sample into a 13 x 100 mm glass test tube.

Step 2: Add 2mL of methylene chloride. Vortex for one minute.

Step 3: Pipette 500µL methylene chloride (bottom layer) into duplicate assay tubes. Evaporate.

Step 4: Dissolve residue in 100µL Calibrator Diluent.

Note: After assaying the extracted sample, the calculated value must be multiplied by the dilution factor.

Materials Provided

1. **CAL** - Calibrator (**REF AC-1501**):

Lyophilised horse serum containing corticosterone and preservative. The exact value of the calibrator is printed on the QC Report. 1 mL per bottle, 1 bottle per kit.

2. **MICROPLAT** - Antibody Coated Plate (**REF AC-1502W**):

Microplate with polyclonal sheep anti-corticosterone antibody linked to the inner surface of the polystyrene wells, 12 x 8-well strips in a foil pouch with desiccant.

3. **ENZYMCONJ** - Enzyme Conjugate (**REF AC-1503**):

An proprietary aqueous solution containing corticosterone labelled with horseradish peroxidase, protein, enzyme stabilisers and preservative. 12 mL per bottle.

4. **CTRL 1** - Control 1 (**REF AC-1505A**):

Lyophilised horse serum containing preservative, 0.5 mL per bottle.

5. **CTRL 2** - Control 2 (**REF AC-1505B**):

Lyophilised horse serum containing rat serum and preservative, 0.5 mL per bottle.

6. **BUF** - Buffer (**REF AC-1507**):

Phosphate buffered saline containing preservative, 21 mL per bottle.

7. **CALDIL** - Calibrator Diluent (**REF AC-1509**):

Corticosterone free horse serum containing preservative, 10 mL per bottle.

8. **TMB** - TMB Substrate (**REF AC-TMB**):

A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 24 mL per bottle.

9. **HCL** - Stop Solution (**REF AC-STOP**):

0.5M hydrochloric acid, 14 mL per bottle.

10. **WASHBUF 20x** - Wash Concentrate (**REF AC-WASHL**):

Phosphate buffered saline containing Tween, 50 mL per bottle.

11. **Adhesive plate sealer.**

4 per kit.

Materials Required but not Provided

1. Disposable 0.5 mL capped micro centrifuge tubes.
2. Precision pipetting devices to deliver 100 μ L and 150 μ L.
3. Repeating pipettes to deliver 400 μ L, e.g. Eppendorf Multipipette 4780, or similar.
4. Precision multi-channel pipettes to deliver 100 μ L and 200 μ L.
5. Heating Water Bath (up to 80°C).
6. Vortex mixer.
7. Automatic microplate washer (optional).
8. Photometric microplate reader and data analysis equipment

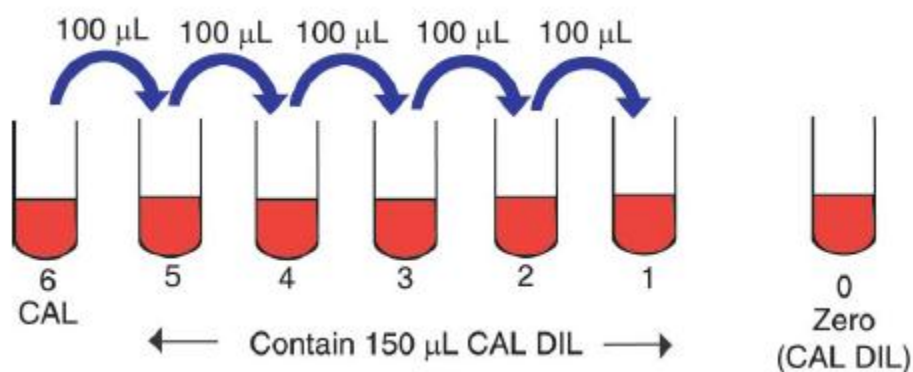
Procedure

Prepare samples as stated in “Specimen Collection, Storage and Preparation” section.

Preparation of Calibrators

1. Label five 0.5 mL micro centrifuge tubes, 1-5.
2. Add 150 μ L of Calibrator Diluent **CALDIL** to all tubes.
3. Add 100 μ L Calibrator **CAL** to tube 5, etc. to produce a dilution series (see table and diagram). Use a fresh pipette tip for each transfer. Vortex each tube thoroughly before the next transfer. Calibrator Diluent serves as the zero calibrator.

Calibrator	Composition
Calibrator 6	CAL
Calibrator 5	150 μ L of Calibrator Diluent CALDIL + 100 μ L Calibrator CAL
Calibrator 4	150 μ L of Calibrator Diluent CALDIL + 100 μ L Calibrator 5
Calibrator 3	150 μ L of Calibrator Diluent CALDIL + 100 μ L Calibrator 4
Calibrator 2	150 μ L of Calibrator Diluent CALDIL + 100 μ L Calibrator 3
Calibrator 1	150 μ L of Calibrator Diluent CALDIL + 100 μ L Calibrator 2
Calibrator 0	Calibrator Diluent CALDIL



Sample Heat Treatment

These volumes are sufficient for duplicate transfers to the microplate.

1. Prepare eppendorf tubes, one for each Calibrator, Control and Sample.
2. Add 100 µL of each Calibrator (0 to 6), Control **CTRL** or Sample to appropriately labelled tubes.
3. Add 400 µL of Buffer **BUF** (blue) to each tube using a repeating pipette. Vortex all tubes. Incubate at 80°C for 30 minutes.
4. Allow samples to cool to 18-25°C, then invert and vortex to ensure all condensation is amalgamated into the sample before use in assay.

Alternative Sample Heat Treatment

Suitable for samples where the volume available is less than 100 µL.

1. Prepare eppendorf tubes, one for each Sample.
2. Add 50 µL of each Sample to appropriately labelled tubes.
3. Add 200 µL of Buffer **BUF** (blue) to each tube using a repeating pipette. Vortex all tubes. Incubate at 80°C for 30 minutes.
4. Allow samples to cool to 18-25°C, then invert and vortex to ensure all condensation is amalgamated into the sample before use in assay

Assay Procedure

1. Add **100 µL** of each heat treated Calibrator, Control or sample to the appropriate wells of the Antibody Coated Plate **MICROPLAT** in duplicate.
2. Add **100 µL** of Enzyme Conjugate **ENZYMCONJ** to all wells using a multichannel pipette.
3. Cover the plate with an adhesive plate sealer and incubate the plate at 18-25°C for 4 hours.
4. Wash all wells three times with Wash Solution.

- a) Automatic plate wash: Set plate washer to dispense at least 300 µL of Wash Solution per well. Fill and aspirate for 3 cycles.
- b) Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µL of Wash Solution to all wells. Decant and repeat twice.

Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution before proceeding to the next step.

5. Add **200 µL** of TMB Substrate **TMB** to all wells using a multichannel pipette.

Note: TMB Substrate is easily contaminated. Measure out the amount of TMB Substrate required for the assay. Do not return unused TMB Substrate to the bottle.

6. Incubate at 18-25°C for 30 minutes.
7. Add **100 µL** of Stop Solution **HCL** to all wells using a multichannel pipette.
8. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results

Calculate the percent binding (B/Bo%) of each Calibrator, Control and unknown sample as follows:

$$B/Bo\% = \frac{\text{(mean absorbance)}}{\text{(mean absorbance for '0' calibrator)}} \times 100$$

Prepare a calibration curve on semi-log graph paper by plotting B/Bo% on the ordinate against concentration of corticosterone on the abscissa. Calculate B/Bo% for each unknown sample and read values off the curve in ng/mL.

Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. Smoothed spline or 4PL curve fits are recommended

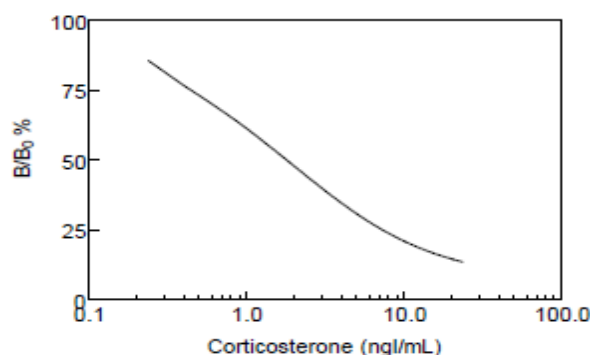
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result. The exact value of the calibrator is printed on the QC Report

Well	Description	Abs.	Mean. Abs.	B/Bo%	Result ng/mL
A1, A2	Calibrator 0 0 ng/mL	2.333 2.410	2.372	100.0	
B1, B2	Calibrator 1 0.24 ng/mL	2.093 1.961	2.027	85.5	
C1, C2	Calibrator 2 0.60 ng/mL	1.668 1.702	1.685	71.1	
D1, D2	Calibrator 3 1.5 ng/mL	1.264 1.222	1.243	52.4	
E1, E2	Calibrator 4 3.8 ng/mL	0.873 0.865	0.869	36.6	
F1, F2	Calibrator 5 9.4 ng/mL	0.527 0.526	0.527	22.2	
G1, G2	Calibrator 6 23.5 ng/mL	0.307 0.322	0.315	13.3	
H1, H2	Sample 1	1.864 1.850	1.857	78.3	0.39
A3, A4	Sample 2	0.664 0.665	0.665	28.0	6.2

Typical Calibration Curve

This sample calibration curve is for illustration o



Limitations of Use

The following substances have been tested and found not to interfere in the Immunodiagnostic Systems Limited Corticosterone (High Sensitivity) EIA:

Haemoglobin	tested up to 100 mg/dL
Bilirubin	tested up to 100 µg/mL
Lipid	tested up to 500 mg/dL triglyceride

Expected Values

Since corticosterone levels can vary greatly due to handling and sampling techniques, it is recommended that each laboratory establish its own normal ranges.

Performance Data

Sensitivity

The sensitivity, defined as the concentration corresponding to the mean minus 2 standard deviations of 20 replicates of the zero calibrator, is 0.17 ng/mL.

Precision

Intra assay mean (ng/mL)	n=20 % CV	Inter assay mean (ng/mL)	n=75 % CV
6.7	7.0	6.4	8.2
17.2	5.9	17.5	8.9

Recovery

Recovery was assessed by adding corticosterone to samples prior to dilution and assay

Sample Concentration ng/ml	Corticosterone added ng/ml	Measured ng/ml	Recovery ng/ml	Recovery %
7.8	5.0	13.4	5.6	112
5.9	5.0	10.6	4.7	94
2.7	5.0	8.2	5.4	109
8.2	5.0	13.6	5.5	109
9.6	5.0	15.4	5.8	116
			Mean	108

Linearity

Linearity was assessed by diluting samples in Calibrator Diluent

Sample	Measured (M) ng/ml	Expected (E) ng/ml	% M/E
Chicken A	7.43	-	-
A/2	3.97	3.71	107
A/4	1.71	1.86	92
A/8	0.88	0.93	95
A/16	0.46	0.46	113
Rat B/40	14.52	-	-
B/80	7.36	7.26	101
B/160	3.77	3.63	104
B/320	1.83	1.81	101
B/640	0.78	0.91	86
		Mean	99

Specificity

The specificity of the antiserum was assessed with the following analytes at 50% binding of the zero calibrator

Analyte	Cross-reactivity
Corticosterone	100
11-Desoxycorticosterone	18.5
11-Dehydrocorticosterone	2.0
Aldosterone	0.26
Dexamethasone	0.11
Cortisol	0.09
Progesterone	0.09
Prednisolone	0.08
11-Desoxycortisol	0.05
Tetrahydrocortisol	0.02
20-b-Hydroxyprogesterone	0.01
Pregnenolone	0.01
Cortisone	0.01
5a-Pregnan-3, 20-dione	0.01
4-Pregen-20-a-ol-3-one	0.01
17a-Hydroxypregnenolone	<0.01
17a-Hydroxyprogesterone	<0.01
Predisone	<0.01
Pregnantriol	<0.01
Tetrahydrocortisone	<0.01

References

1. Corker C.S. Karolinska Symposia on Research Methods in Reproductive Endocrinology : Steroid Assay by Protein Binding. E. Diczfalusy, ed., p 153 (1970).
2. Keith L. D., Winslow J. R. and Reynolds R. W. Steroids, 31: 523 (1978).
3. Shimizu K., Amagaya S. and Ogihara Y. : Analysis of Corticosterone in the serum of mice and rats, using high performance liquid chromatography. J. Chromatography, 272: 170
4. Vining R.F., James D. E., Bennett S. P. and Kraegen E. W. Steroids, 38: 297 (1981)

	GB <i>Use By</i> DE <i>Verwendbar bis</i> ES <i>Fecha de caducidad</i> IT <i>Utilizzare entro</i> FR <i>Utiliser jusque</i> NL <i>Houdbaar tot</i> DK <i>Holdbar til</i> CZ <i>Použitelné do</i> SK <i>Použitelné do</i> GR <i>Ημερομηνία λήξης</i> PT <i>Prazo de validade</i> HU <i>Felhasználható</i> SE <i>Använd före</i> PL <i>Użyć przed</i>	LOT	GB <i>Batch code</i> DE <i>Chargenbezeichnung</i> ES <i>Código de lote</i> IT <i>Codice del lotto</i> FR <i>Code du lot</i> NL <i>Lot nummer</i> DK <i>Lotnummer</i> CZ <i>Číslo šarže</i> SK <i>Číslo šarže</i> GR <i>Αριθμός Παρτίδας</i> PT <i>Código do lote</i> HU <i>Sarzszzám</i> SE <i>Lot nummer</i> PL <i>Kod partii</i>
REF	GB <i>Catalogue number</i> DE <i>Bestellnummer</i> ES <i>Número de catálogo</i> IT <i>Numero di catalogo</i> FR <i>Référence du catalogue</i> NL <i>Catalogus nummer</i> DK <i>Katalognummer</i> CZ <i>Katalogové číslo</i> SK <i>Katalógové číslo</i> GR <i>Αριθμός καταλόγου</i> PT <i>Referência de catálogo</i> HU <i>Katalógusszám</i> SE <i>Katalognummer</i> PL <i>Numer katalogowy</i>		GB <i>Manufacturer</i> DE <i>Hersteller</i> ES <i>Fabricante</i> IT <i>Fabbricante</i> FR <i>Fabricant</i> NL <i>Fabrikant</i> DK <i>Producent</i> CZ <i>Výrobce</i> SK <i>Výrobca</i> GR <i>Κατασκευαστής</i> PT <i>Fabricante</i> HU <i>Gyártó</i> SE <i>Tillverkare</i> PL <i>Producent</i>
	GB <i>Contains sufficient for <n> tests</i> DE <i>Inhalt ausreichend für <n> Prüfungen</i> ES <i>Contenido suficiente para <n> ensayos</i> IT <i>Contenuto sufficiente per "n" saggi</i> FR <i>Contenu suffisant pour "n" tests</i> NL <i>Inhoud voldoende voor "n" testen</i> DK <i>Indeholder tilstrækkeligt til "n" test</i> CZ <i>Lze použít pro <n> testů</i> SK <i>Obsah postačuje na <n> stanovení</i> GR <i>Περιεχόμενο επαρκές για «ν» εξετάσεις</i> PT <i>Conteúdo suficiente para "n" ensaios</i> HU <i>A doboz tartalma <n> vizsgálat elvégzéséhez elegendő</i> SE <i>Räcker till "n" antal tester</i> PL <i>Wystarczy na wykonanie <n> testów</i>	IVD	GB <i>In Vitro Diagnostic Medical Device</i> DE <i>In-Vitro-Diagnostikum</i> ES <i>Producto sanitario para diagnóstico in vitro</i> IT <i>Dispositivo medico-diagnostico in vitro</i> FR <i>Dispositif médical de diagnostic in vitro</i> NL <i>Medisch hulpmiddel voor in-vitro diagnostiek</i> DK <i>Medicinsk udstyr til in vitro-diagnostik</i> CZ <i>In Vitro diagnostický zdravotnický prostředek</i> SK <i>Zdravotnícka pomôcka in vitro</i> GR <i>Ιn Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν</i> PT <i>Dispositivo médico para diagnóstico in vitro</i> HU <i>In vitro diagnosztikum</i> SE <i>Medicintekniska produkter för in vitro diagnostik</i> PL <i>Wyrób do diagnostyki In Vitro</i>
	GB <i>Temperature limitation</i> DE <i>Temperaturbegrenzung</i> ES <i>Límite de temperatura</i> IT <i>Limiti di temperatura</i> FR <i>Limites de température</i> NL <i>Temperatuurlimiet</i> DK <i>Temperaturbegrænsning</i> CZ <i>Teplotní rozmezí od do</i> SK <i>Teplotné rozmedzie od do</i> GR <i>Περιορισμοί θερμοκρασίας</i> PT <i>Limites de temperatura</i> HU <i>Hőmérséklettartomány</i> SE <i>Temperaturbegränsning</i> PL <i>Przestrzegać zakresu temperatury</i>		GB <i>Consult Instructions for Use</i> DE <i>Gebrauchsanweisung beachten</i> ES <i>Consulte las instrucciones de uso</i> IT <i>Consultare le istruzioni per l'uso</i> FR <i>Consulter les instructions d'utilisation</i> NL <i>Raadpleeg de gebruiksaanwijzing</i> DK <i>Se brugsanvisning</i> CZ <i>Viz návod k použití</i> SK <i>Viď návod na použitie</i> GR <i>Συμβουλευτείτε τις οδηγίες χρήσης</i> PT <i>Consulte as instruções de utilização</i> HU <i>Nézz meg a Használati utasítást</i> SE <i>Se handhavandebeskrivningen</i> PL <i>Sprawdź w instrukcji obsługi</i>

Procedure Summary

SAMPLE HEAT TREATMENT

100 µL Calibrator, Control or sample.

Add 400 µL Buffer **BUF**. Vortex



Incubate: 30 minutes @ 80°C



Allow to cool to 18-25°C. Vortex

ASSAY

Add 100 µL heat treated Calibrator, Control or sample

to Antibody Coated Plate **MICROPLAT**

Add 100 µL Enzyme Conjugate **ENZYMCONJ**



Incubate: 4 hours @ 18-25°C



Wash plate.



Add 200 µL TMB Substrate **TMB**



Incubate: 30 minutes @ 18-25°C



Add 100 µL Stop Solution **HCL**



Read plate @ 450nm.



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