immunodiagnosticsystems

Corticosterone EIA

Enzymeimmunoassay for the quantitative determination of corticosterone in mouse and rat serum or plasma

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

REF AC-14F1 Σ 96

Intended Use

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

The IDS Corticosterone EIA kit is a competitive enzymeimmunoassay (EIA) for the quantitative determination of corticosterone in mouse and rat serum or plasma.

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 rats and mice. The majority of corticosterone in the circulation is carried by corticosteroid binding globulin. The level of corticosterone in rats and mice is of the same order of magnitude as that of cortisol in the human, and 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 IDS Corticosterone EIA kit is a competitive enzymeimmunoassay utilising a polyclonal corticosterone antibody coated onto the inner surface of polystyrene microtitre wells. Calibrators, Controls and diluted samples are incubated with enzyme (horseradish peroxidase) labelled corticosterone in the antibody coated wells overnight at 2 8°C. 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 IDS Corticosterone 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. IDS 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 human and/or 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.

0.5M hydrochloric acid

Stop Solution HCL contains 0.5M hydrochloric acid.
R36/38 Irritating to eyes and skin.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.

Tetramethylbenzidine

TMB Substrate SUBS contains 3,3',5,5'-tetramethylbenzidine.
R21/22 Harmful by contact with skin and if swallowed.
S36/37 Wear suitable protective clothing and gloves.

Preparation of Reagents

Calibrators CAL and Controls CTRL : Calibrators CAL and Controls CTRL are 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.

If Calibrators or Controls are to be used more than once they must be frozen (-20°C) as soon as possible after reconstitution. When reusing Calibrators and Controls, thaw at room temperature, mix and use within 15 minutes of completion of thaw.

Enzyme Conjugate Solution: Enzyme Conjugate ENZYMCONJ is supplied lyophilised. Add the contents of the bottle of Buffer BUF to the bottle of lyophilised Enzyme Conjugate ENZYMCONJ. Replace the stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution.

If Enzyme Conjugate Solution is to be used more than once it must be frozen (-20°C) as soon as possible after reconstitution. When reusing, thaw at room temperature, mix and use within 15 minutes of completion of thaw.

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 Calibrators CAL, Controls CTRL and Enzyme Conjugate Solution can be stored at -20°C for up to 8 weeks.

Unused Antibody Coated Plate strips must be returned to the foil pouch with the desiccant sachet. Fold over the end of the foil pouch and seal in one of the plastic selfseal bags provided. Store at 2-8°C for up to 8 weeks.

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

Indications of possible deterioration of kit reagents

The presence of abnormal particulate matter in any of the reagents, excluding the Sample Diluent SAMPDIL.

A decrease in the absorbance of the zero calibrator.

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

Specimen Collection and Storage

The assay should be performed using serum or plasma (EDTA, heparin or citrate) 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.

Procedure

Materials Provided

1. CAL 0-6 - **Calibrators (REF AC-1401A - AC-1401G):**

   Lyophilised phosphate buffered saline containing corticosterone, protein and preservative. 1 mL per bottle, 7 bottles per kit.

2. MICROPLAT - **Antibody Coated Plate (REF AC-1402W):**

   Microplate with polyclonal rabbit 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-1403):**

   Lyophilised phosphate buffered saline containing corticosterone labelled with horseradish peroxidase, protein, enzyme stabilisers and preservative. 2 mL per bottle.

4. BUF - **Buffer (REF AC-1403B):**

   Phosphate buffered saline containing preservative, 12 mL per bottle.

5. CTRL 1-2 - **Controls (REF AC-1405A - AC-1405B)**:

   Lyophilised mouse or rat serum pre-diluted in Sample Diluent, 1 mL per bottle, 2 bottles per kit.

6. SUBS - **TMB Substrate (REF AC-SUBS**):

   A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 30 mL per bottle.

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

   0.5M hydrochloric acid, 14 mL per bottle.

8. SAMPDIL - **Sample Diluent (REF AC-1400B)**:

   Phosphate buffered saline containing horse serum, protein and preservative, 15 mL per bottle.
   The Sample Diluent may contain a light precipitate. Mix well before use.

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

   Phosphate buffered saline containing Tween, 50 mL per bottle.

10. **Adhesive plate sealer**.

    4 per kit.

Materials Required but not Provided

1. Disposable 12 x 75 mm borosilicate glass, polypropylene or polystyrene tubes. Do not reuse tubes.
2. Precision pipetting devices to deliver 30 µL, 100 µL and 270 µL.
3. Precision multi-channel pipettes to deliver 100 µL and 200 µL.
4. Vortex mixer.
5. Automatic microplate washer (optional).
6. Photometric microplate reader and data analysis equipment.

Sample Dilution

DO NOT dilute Calibrators [CAL] or Controls [CTRL]

1. Prepare labelled tubes, one for each sample.
2. Dilute each sample at least one in ten using Sample Diluent [SAMPDIL]

   For example:

   Add **30 µL** of each unknown sample to appropriately labelled tubes.

   Add **270 µL** of Sample Diluent [SAMPDIL] to each tube.
3. Vortex all tubes.

Assay Procedure

1. Add **100 µL** of each Calibrator [CAL], Control [CTRL] or diluted sample to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.
2. Add **100 µL** of Enzyme Conjugate Solution to all wells using a multichannel pipette.
3. Cover the plate with an adhesive plate sealer and incubate at 2-8°C for 16-24 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 [SUBS] 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.

To obtain the concentration of corticosterone in each sample, multiply the value read from the curve by the dilution factor used.

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

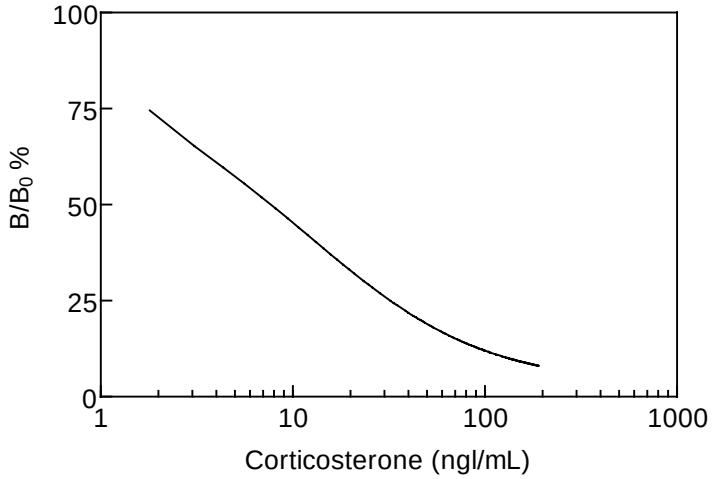
Sample Assay Data

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

Well	Description	Abs.	Mean. Abs.	B/Bo%	Result ng/mL
A1, A2	Calibrator 0 0 ng/mL	2.172 2.183	2.178		
B1, B2	Calibrator 1 1.8 ng/mL	1.533 1.577	1.555	71.4	
C1, C2	Calibrator 2 4.2 ng/mL	1.133 1.260	1.197	56.0	
D1, D2	Calibrator 3 10.6 ng/mL	0.761 0.812	0.787	36.1	
E1, E2	Calibrator 4 28.2 ng/mL	0.478 0.476	0.477	21.9	
F1, F2	Calibrator 5 75.0 ng/mL	0.277 0.266	0.272	12.5	
G1, G2	Calibrator 6 186 ng/mL	0.159 0.154	0.157	7.2	
H1, H2	Sample 1	1.104 0.963	1.034	47.5	6.3
A3, A4	Sample 2	0.422 0.410	0.416	19.1	33.3

Typical Calibration Curve

This sample calibration curve is for illustration only.



Limitations of Use

The following substances have been tested and found not to interfere in the IDS Corticosterone EIA:

Haemoglobin tested up to 1470 mg/dL

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. As a guide 16 normal rat/mouse serum/plasma samples ranged from 23 to 363 ng/mL.

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 0.55 ng/mL.

Precision

Intra assay mean (ng/mL)	n=10 % CV	Inter assay mean (ng/mL)	n=25 % CV
4.6	4.9%	4.7	7.8%
10.8	6.6%	10.5	8.6%
20.6	4.5%	19.3	7.5%
45.7	3.8%	45.2	7.7%

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 %
64	200	257	193	97%
506	100	628	122	122%
55	200	224	169	85%
65	200	247	182	91%
430	200	626	196	98%
			Mean	**99%**

Linearity

Linearity was assessed by diluting samples in Sample Diluent.

Sample	Measured (M) ng/ml	Expected (E) ng/ml	% M/E
A/10	36.1		
A/20	16.8	18.1	93%
A/40	8.1	9.0	90%
B/10	13.3		
B/20	7.2	6.7	107%
B/40	3.9	3.3	118%
C/10	30.8		
C/20	14.8	15.4	96%
C/40	7.2	7.7	94%
		Mean	**100%**

Specificity

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

Analyte	Cross-reactivity
11-Dehydrocorticosterone	6.60%
11-Deoxycorticosterone	5.93%
Progesterone	1.39%
Cortisol	0.85%
Prednisolone	0.60%
21-Deoxycortisol	0.34%
5α-Pregnan-3, 20-dione	0.21%
Tetrahydrocortisone	<0.07%
Dexamethasone	0.07%
DHEA	<0.07%
Prednisone	<0.07%
Pregnantriol	<0.07%
20β-Hydroxyprogesterone	<0.07%
4-Pregnen-20α-ol-3-one	<0.06%
Oestriol	<0.06%
Oestradiol	<0.06%
Oestrone	<0.06%
Pregnenolone	0.06%
17α-Hydroxypregnolone	<0.05%
Cortisone	0.05%
Testosterone	0.02%
11-Desoxycortisol	0.02%
Aldosterone	0.02%
17α-Hydroxyprogesterone	0.01%
Tetrahydrocortisol	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).
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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)

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	GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Temperature limitation* *Temperaturbegrenzung* *Límite de temperatura* *Limiti di temperatura* *Limites de température* *Temperatuurlimiet* *Temperaturbegrænsning* *Teplotní rozmezí od do* *Teplotné rozmedzie od do* *Περιορισμοί θερμοκρασίας* *Limites de temperatura* *Hőmérséklettartomány* *Temperaturbegränsning* *Przestrzegać zakresu temperatury*		GB DE ES IT FR NL DK CZ SK GR PT HU SE PL	*Consult Instructions for Use* *Gebrauchsanweisung beachten* *Consulte las instrucciones de uso* *Consultare le istruzioni per l'uso* *Consulter les instructions d'utilisation* *Raadpleeg de gebruiksaanwijzing* *Se brugsanvisning* *Viz návod k použití* *Viď návod na pužitie* *Συμβουλευτείτε τις οδηγίες χρήσης* *Consulte as instruções de utilização* *Nézze meg a Használati utasítást* *Se handhavandebeskrivningen* *Sprawdź w instrukcji obsługi*

Procedure Summary

SAMPLE DILUTION

30 µL sample.

Add 270 µL Sample Diluent **SAMPDIL**. Vortex.

ASSAY

Add 100 µL Calibrator **CAL**, Control **CTRL** or diluted sample

to Antibody Coated Plate **MICROPLAT**

Add 100 µL Enzyme Conjugate Solution.

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Incubate: 16-24 hours @ 2-8°C

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Wash plate.

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Add 200 µL TMB Substrate **SUBS**

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Incubate: 30 minutes @ 18-25°C

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Add 100 µL Stop Solution **HCL**

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Read plate @ 450nm.

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