Version 12-01/25

User's Manual

Corticosterone ELISA Kit

REF **DEIABL244**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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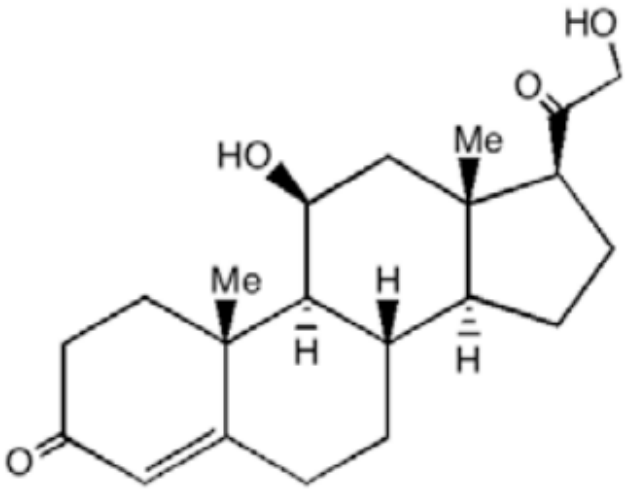
PRODUCT INFORMATION

Intended Use

The Corticosterone ELISA Kit, with its adaptable format and high sensitivity, is a valuable tool for research in endocrinology, stress physiology, and neurobiology. It provides precise measurement of corticosterone levels, aiding in the study of its effects on physiological and behavioral responses.

General Description

Corticosterone ($C_{21}H_{30}O_4$, Kendall's Compound 'B') is a glucocorticoid secreted by the cortex of the adrenal gland. Corticosterone is produced in response to stimulation of the adrenal cortex by ACTH and is the precursor of aldosterone. Corticosterone is a major indicator of stress and is the major stress steroid produced in non-human mammals. Studies involving corticosterone and levels of stress include impairment of long term memory retrieval, chronic corticosterone elevation due to dietary restrictions and in response to burn injuries. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns.



Principles of Testing

The Corticosterone kit is designed to quantitatively measure Corticosterone present in serum, plasma, saliva, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total corticosterone in serum and plasma and in extracted fecal samples.

The kit offers two standard curve ranges. Depending on the anticipated sensitivity needed for your samples, you can use 50 µL of standads and samples with an assay range of 10,000 pg/mL to 39.063 pg/mL or use 100 µL of standards and samples with an assay range of 5,000 to 19.53 pg/mL. A corticosterone stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Please choose the standard range that ffts your sample concentrations most appropriately.

Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture

sheep antibodies. A corticosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to corticosterone to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound corticosterone-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the corticosterone in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

Reagents And Materials Provided

1. Coated Clear 96 Well Plates:

A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

2. Corticosterone Standard:

Corticosterone at 100,000 pg/mL in a special stabilizing solution. 125 µL

3. Corticosterone Antibody:

A sheep polyclonal antibody speciffc for corticosterone. 3 mL

4. Corticosterone Conjugate:

A corticosterone-peroxidase conjugate in a special stabilizing solution. 3 mL

5. Assay Buffer Concentrate:

A 5× concentrate that must be diluted with deionized or distilled water. 28 mL

6. Dissociation Reagent:

Dissociation Reagent is to be used only with Serum and Plasma samples. 1 mL

7. Wash Buffer Concentrate:

A 20× concentrate that should be diluted with deionized or distilled water. 30 mL

8. TMB Substrate:

11 mL

9. Stop Solution:

A 1M solution of hydrochloric acid. CAUSTIC. 5 mL

10. Plate Sealer:

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Materials Required But Not Supplied

Distilled or deionized water.

Organic solvent for fecal extraction and equipment for drying down (such as SpeedVac centrifugal concentrators).

Repeater pipet with disposable tips capable of dispensing 25, 50, and 100 µL.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

Specimen Collection And Preparation

1. SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, saliva and urine samples and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing

visible particulate should be centrifuged prior to using. **Moderate to severely hemolyzed samples should not be used in this kit.**

Corticosterone is identical across all species and we expect this kit may measure corticosterone from sources other than human. The end user should evaluate recoveries of corticosterone in other samples being tested.

2. SAMPLE PREPARATION

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total corticosterone concentration in serum or plasma. Dissociation Reagent is to be used only with Serum and Plasma samples.

a. Serum and Plasma Samples

Allow the Dissociation Reagent to warm completely to Room Temperature before use. We suggest pipetting 5 µL of Dissociation Reagent into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the Dissociation Reagent in the tube, vortex gently and incubate at room temperature for 5 minutes or longer.

Dilute with 490 µL of 1X Assay Buffer. This 1:100 dilution can be diluted further with 1× Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100.

NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

b. Saliva Samples

Saliva samples should be diluted ≥ 1:2 with 1× Assay Buffer prior to running in the assay. You can ask CD for Saliva

Sample Handling instructions.

c. Urine Samples

Urine samples should be diluted ≥ 1:20 with 1× Assay Buffer prior to running in the assay. You can ask CD for Urine

Sample Handling instructions.

d. Dried Fecal Samples

The ethanol concentration in the final 1× Assay Buffer dilution added to the well should be < 5%. You can ask CD for Dried Fecal Samples Handling instructions.

e. Tissue Culture Media

For measuring corticosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.

Use all Samples within 2 hours of preparation, or stored at ≤ -20°C until assaying.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute Assay Buffer Concentrate 5-fold by adding one part of the concentrate to four parts of deionized water.

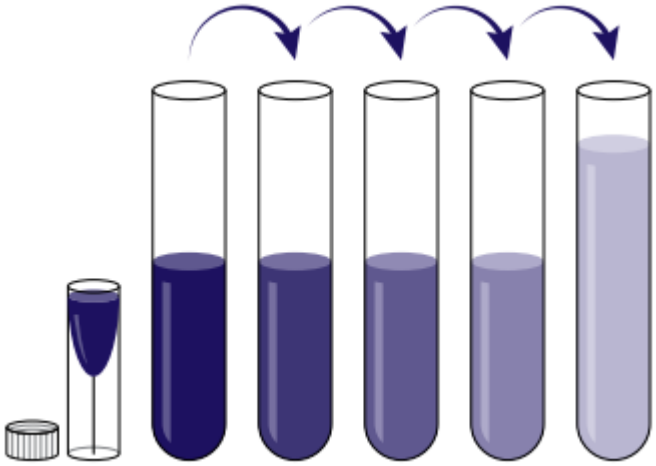
The 1× Assay Buffer is stable for 3 months at 4°C.

Wash Buffer

Dilute Wash Buffer Concentrate 20-fold by adding one part of the concentrate to nineteen parts of deionized water. The 1× Wash Buffer is stable for 3 months at room temperature.

Standard Preparation - 50 µL Assay Format

Label test tubes as #1 through #9. Pipet 450 µL of 1× Assay Buffer into tube #1 and 250 µL into tubes #2 to #9. The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 250 µL of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of corticosterone in tubes 1 through 9 will be 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125 and 39.063 pg/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
1X Assay Buffer (µL)	**450**	250	250	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Vol of Addition (µL)	**50**	250	250	250	250	250	250	250	250
Final Conc (pg/mL)	10,000	5,000	2,500	1,250	625	312.5	156.25	78.125	39.063

Standard Preparation - 100 µL Assay Format

Label test tubes as #1 through #9. Pipet 570 µL of 1× Assay Buffer into tube #1 and 300 µL into tubes #2 to

#9. The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 30 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 300 µL of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of corticosterone in tubes 1 through 9 will be 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125, 39.063 and 19.531 pg/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
1X Assay Buffer (µL)	**570**	300	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Vol of Addition (µL)	**30**	300	300	300	300	300	300	300	300
Final Conc (pg/mL)	5,000	2,500	1,250	625	312.5	156.25	78.125	39.063	19.531

Use all Standards within 2 hours of preparation.

Assay Procedure

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine corticosterone concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4°C. Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL (100 µL for alternative format) of samples or standards into wells in the plate.
4. Pipet 75 µL (125 µL for alternative format) of 1× Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 50 µL (100 µL for alternative format) of 1× Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the Corticosterone Conjugate to each well using a repeater pipet.
7. Add 25 µL of the Corticosterone Antibody to each well, except the NSB wells, using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 1 hour. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 45% lower.
9. Aspirate the plate and wash each well 4 times with 300 µL 1× Wash Buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate corticosterone concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

Calculation

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD' s for the NSB. The sample concentrations obtained, calculated from the % B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

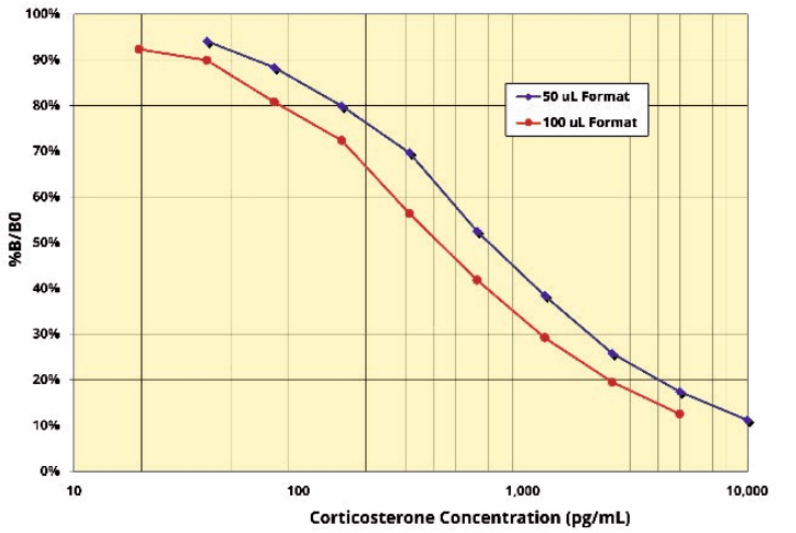
Typical Standard Curve

	50 µL Assay				100 µL Assay			
Sample	Mean OD	Net OD	% B/B0	Corticosterone. Conc. (pg/mL)	Mean OD	Net OD	% B/B0	Corticosterone. Conc. (pg/mL)
NSB	0.087	0	-	-	0.094	0	-	-
Standard 1	0.222	0.135	11.2	10,000	0.210	0.116	12.6	5,000
Standard 2	0.297	0.210	17.4	5,000	0.274	0.180	19.5	2,500
Standard 3	0.398	0.311	25.8	2,500	0.364	0.270	29.3	1,250
Standard 4	0.550	0.463	38.5	1,250	0.480	0.386	41.9	625
Standard 5	0.719	0.632	52.5	625	0.614	0.520	56.4	312.5
Standard 6	0.926	0.839	69.7	312.5	0.761	0.667	72.3	156.3
Standard 7	1.049	0.962	79.9	156.3	0.839	0.745	80.8	78.13
Standard 8	1.151	1.064	88.4	78.13	0.923	0.829	89.9	39.06
Standard 9	1.220	1.133	94.1	39.06	0.945	0.851	92.3	19.53
B0	1.291	1.204	100.0	0	1.016	0.922	100.0	0
Sample 1	0.437	0.350	29.1	2051.74	0.441	0.347	37.6	776.2
Sample 2	0.863	0.776	64.4	379.29	0.595	0.501	54.3	357.0

Always run your own standard curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of corticosterone is equivalent to 288.6 pM.

Typical Normal Range Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

Precision

Intra Assay Precision - 50 µL Assay Format

Four human samples were diluted with 1× Assay Buffer and run in replicates of 20 in an assay. The mean and

precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Conc. (pg/mL)	%CV
1	2,460.6	6.3
2	601.5	6.5
3	371.6	3.1
4	259.0	4.8

Inter Assay Precision - 50 µL Assay Format

Three human samples were diluted with 1× Assay Buffer and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Conc. (pg/mL)	%CV
1	2,618.3	7.5
2	630.1	6.4
3	267.9	9.9

Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #9. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. Sensitivity was determined as 20.9 pg/mL for 50 µL and 14.35 pg/mL for 100 µL sample size.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample. Limit of Detection was determined as 17.5 pg/mL for 50 µL and 7.7 pg/mL for 100 µL sample size.

Specificity

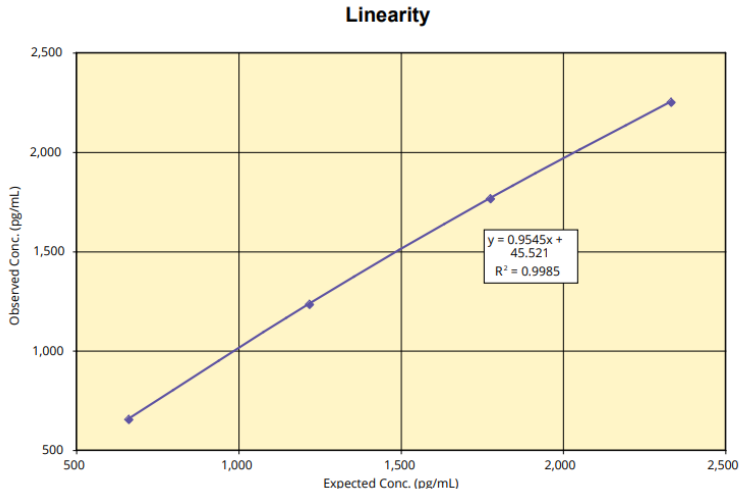
The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
Corticosterone	100%	Testostrone	0.03%
1-dehydrocorticosterone	18.9%	Corticosterone-21-hemisuccinate	< 0.1%
Desoxycorticosterone	12.3%	Cortisone	< 0.08%
1α-hydroxycorticosterone	3.3%	Estradiol	< 0.08%
11-dehydrocorticosterone	2.4%	17-hydroxyprogesterone	< 0.01%
Tetrahydrocorticosterone	0.76%	Allopregnanolone	< 0.01%
Aldosterone	0.62%	Dehydroepiandrosterone sulfate	< 0.01%
Cortisol	0.38%	Estrone-3-glucuronide	< 0.01%
Progesterone	0.24%	Estrone-3-sulfate	< 0.01%
Dexamethasone	0.12%		

Linearity

Linearity was determined in 50 µL assay format by taking two serum samples treated with Dissociation Reagent and diluted 1:50 with 1× Assay Buffer, one with a low diluted corticosterone level of 104.6 pg/mL and one with a higher diluted level of 2,890.5 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Serum	High Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	661.8	654.0	98.8
60%	40%	1,219.0	1,232.3	101.1
40%	60%	1,776.1	1,763.9	99.3
20%	80%	2,333.3	2,249.5	96.4
			Mean Recovery	**98.9%**



Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.