

**KAMIYA BIOMEDICAL COMPANY**

# Corticosterone Chemiluminescent CLIA kit

**For the quantitative determination of corticosterone in  
dried fecal extracts, serum, EDTA and heparin plasma and tissue culture media**

**Cat. No. KT-714**

**For Research Use Only.**

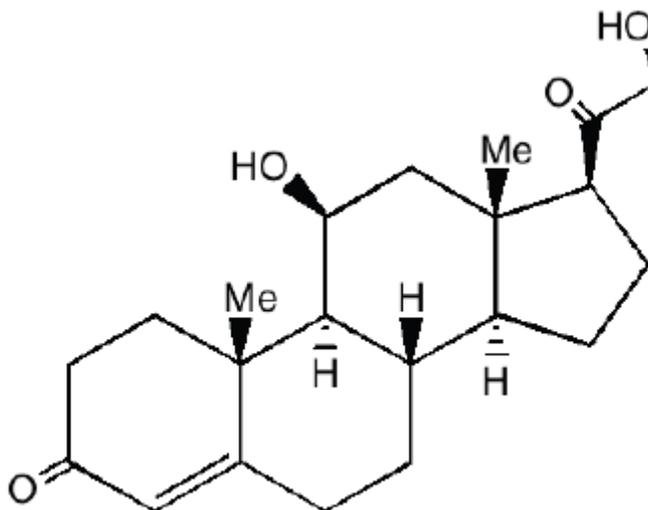
## PRODUCT INFORMATION

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#### BACKGROUND

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.



#### PRINCIPLE

The Corticosterone Immunoassay kit is designed to quantitatively measure Corticosterone present in extracted dried fecal samples, serum, plasma 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. A corticosterone calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators or diluted samples are pipetted into a white microtiter plate coated with an antibody to capture sheep antibodies. A corticosterone-peroxidase conjugate is added to the calibrators and samples in the wells. The binding reaction is initiated by the addition of a sheep polyclonal antibody to corticosterone to each well. After a two hour incubation the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound corticosterone-peroxidase conjugate to produce light. The generated light is detected in a microtiter plate reader capable of reading luminescence. 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.

#### COMPONENTS

##### Coated White 96 Well Plates

White plastic break-apart strip microtiter plate(s) coated with donkey anti-sheep IgG.

1 Each

##### Corticosterone Calibrator

Corticosterone at 50,000 pg/mL in a special stabilizing solution.

125  $\mu$ L

**Corticosterone CLIA Antibody**

A sheep polyclonal antibody specific for corticosterone.

3 mL

**Corticosterone CLIA Conjugate**

A corticosterone-peroxidase conjugate in a special stabilizing solution.

3 mL

**Assay Buffer (or Concentrate)**

One plate kit uses a ready-to-use Assay Buffer. Five plate kit uses a 5X concentrate that should be diluted with deionized or distilled water.

28 mL

**Dissociation Reagent**

1 mL

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

**Wash Buffer Concentrate**

A 20X concentrate that should be diluted with deionized or distilled water.

30 mL

**Substrate Solution A**

6 mL

**Substrate Solution B**

6 mL

**Plate Sealer**

1 Each

**STORAGE**

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

**OTHER MATERIALS REQUIRED**

Distilled or deionized water.

Microplate shaker.

Repeater pipet with disposable tips capable of dispensing 25  $\mu$ L and 100  $\mu$ L.

96 well microplate reader capable of reading glow chemiluminescence. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. **The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:**

Dilute 5  $\mu$ L of the Corticosterone Conjugate into 995  $\mu$ L of deionized water. Pipet 5  $\mu$ L of diluted conjugate into a white well and add 100  $\mu$ L of prepared CLIA substrate. This well will give you an intensity slightly above the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the maximum signal.

**To properly analyze the data, software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.**

**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.

## SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma 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.

## 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.**

### Serum and Plasma Samples

Allow the Dissociation Reagent (DR) to warm completely to **Room Temperature** before use. We suggest pipeting 5  $\mu\text{L}$  of DR into 1 mL Eppendorf tubes. Add 5  $\mu\text{L}$  of serum or plasma to the DR in the tube, vortex gently and incubate at room temperature for 5 minutes or longer. Dilute with 490  $\mu\text{L}$  of supplied Assay Buffer. This 1:100 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be  $\geq 1:100$ .

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

### Dried Fecal Samples

The ethanol concentration in the final Assay Buffer dilution added to the well should be  $<5\%$ .

### Tissue Culture Media

For measuring corticosterone in tissue culture media (TCM), samples should be read off a calibration curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

**Use all Samples within 2 Hours of preparation, or stored at  $\leq -20^{\circ}\text{C}$  until assaying.**

## REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine corticosterone concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

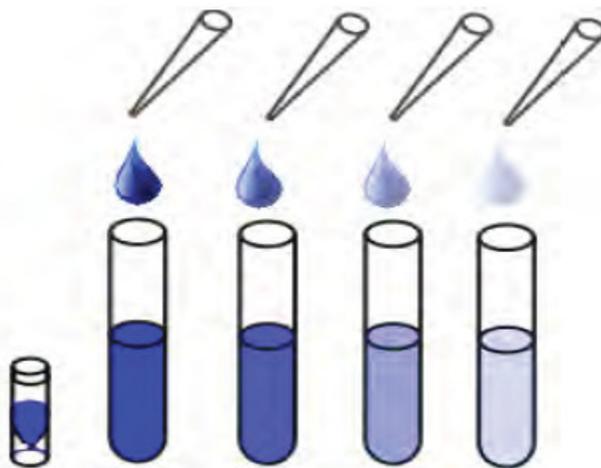
### Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.

### Calibrator Preparation

Label seven test tubes as #1 through #7. Pipet 470  $\mu\text{L}$  of Assay Buffer into tube #1 and 225  $\mu\text{L}$  into tubes #2 to #7. **The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 30  $\mu\text{L}$  of the corticosterone stock solution to tube #1 and vortex completely. Take 150  $\mu\text{L}$  of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of corticosterone in tubes 1 through 7 will be 3,000, 1,200, 480, 192, 76.8, 30.72, and 12.288  $\mu\text{g/mL}$ .

**Use all Calibrators within 2 hour of preparation.**



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
<b>Assay Buffer (μL)</b>	<b>470</b>	225	225	225	225	225	225
<b>Addition</b>	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
<b>Vol of Addition (μL)</b>	<b>30</b>	150	150	150	150	150	150
<b>Final Conc (pg/mL)</b>	3,000	1,200	480	192	76.8	30.72	12.288

#### Chemiluminescent Substrate

Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

	1 Plate	2 Plates	3 Plates	4 Plates	5 Plates
<b>Substrate A &amp; B</b>	5 mL	10 mL	15 mL	20 mL	25 mL
<b>Final Mixture</b>	10 mL	20 mL	30 mL	40 mL	50 mL

## ASSAY PROTOCOL

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. 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.
2. Pipet 50 μL of samples or calibrators into wells in the plate.
3. Pipet 75 μL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 μL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).
5. Add 25 μL of the Corticosterone CLIA Conjugate to each well using a repeater pipet.
6. Add 25 μL of the Corticosterone CLIA Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. 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 2 hours. If the plate is not shaken signals bound will be approximately 45% lower.
8. Aspirate the plate and wash each well 4 times with 300 μL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 μL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 5 minutes without shaking.

11. Read the luminescence generated from each well in a multimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.

12. Use the plate reader's built-in 4PLC software capabilities to calculate corticosterone concentration for each sample.

**CALCULATION OF RESULTS**

Average the duplicate RLU readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean RLUs 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.

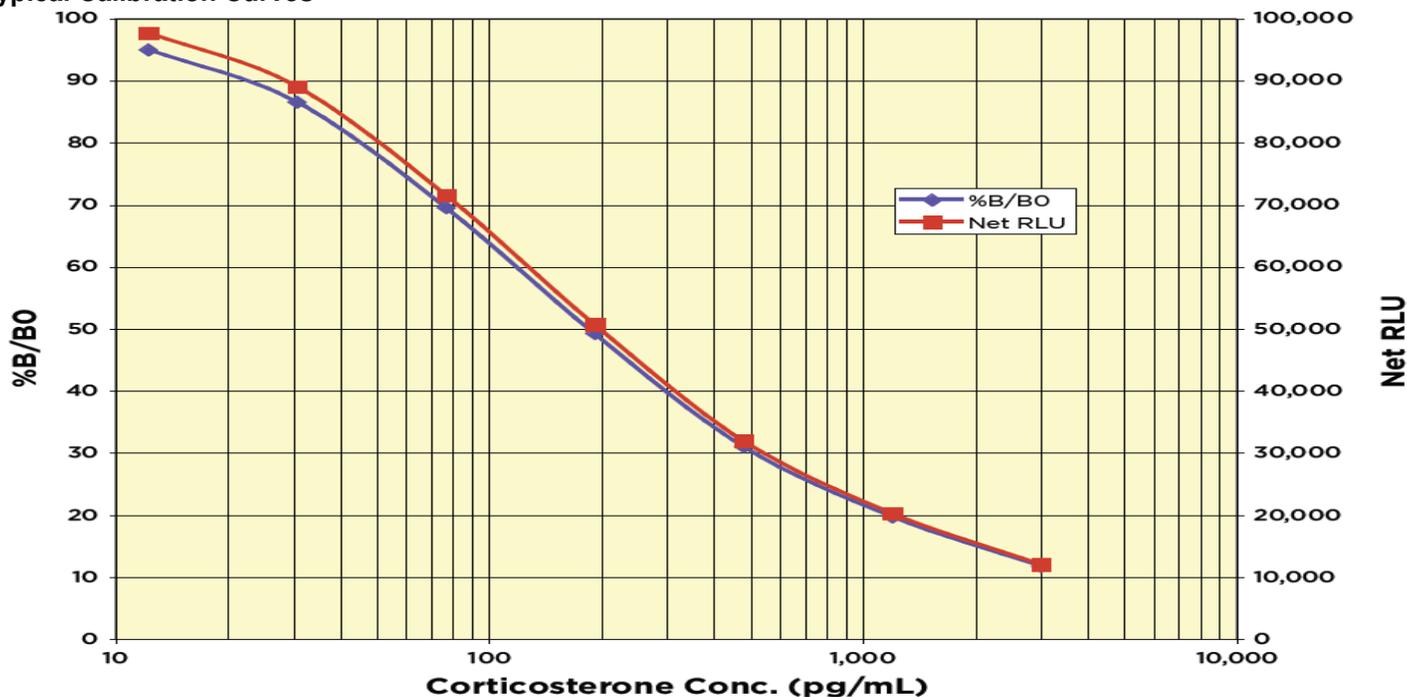
**TYPICAL DATA**

Sample	Mean RLU	Net RLU	% B/B0	Corticosterone Conc. (pg/mL)
NSB	10,460	0	-	-
Standard 1	22,450	11,990	11.7	3,000
Standard 2	30,680	20,220	19.7	1,200
Standard 3	42,385	31,925	31.0	480
Standard 4	61,120	50,660	49.2	192
Standard 5	81,970	71,510	69.5	76.8
Standard 6	99,435	88,975	86.5	30.72
Standard 7	108,055	97,595	94.9	12.288
B0	113,325	102,865	100.0	0
Sample 1	57,960	47,500	46.18	224.8
Sample 2	85,865	75,405	73.30	64.43

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

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

**Typical Calibration Curves**



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

**VALIDATION DATA**

**Sensitivity and Limit of Detection**

Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and calibrator #7. The detection limit was determined at two (2) standard deviations from the B0 along the calibration curve.

**Sensitivity was determined as 6.71 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty wells run for each of the zero calibrator and a low concentration mouse sample.

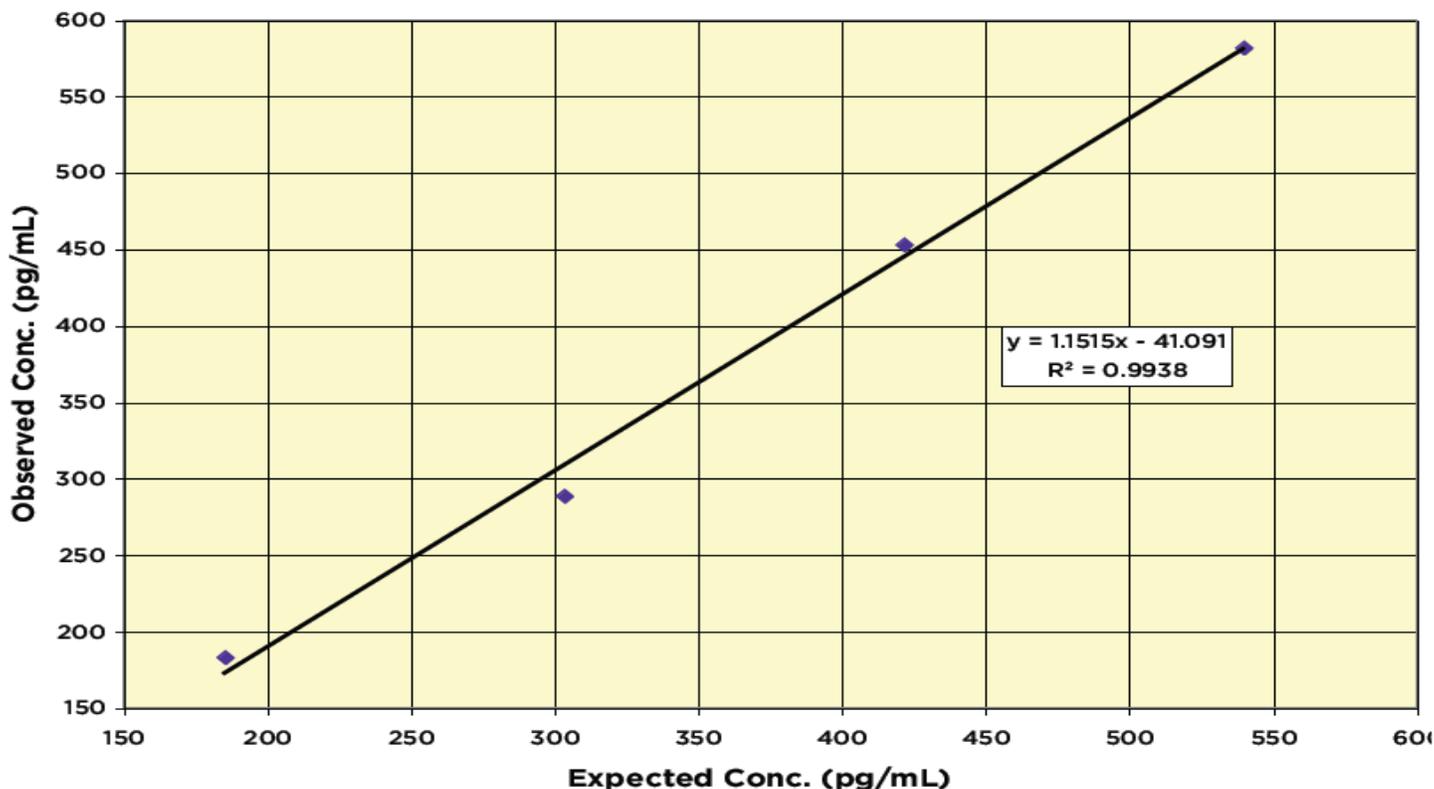
**Limit of Detection was determined as 12.8 pg/mL.**

**Linearity**

Linearity was determined by taking two mouse plasma samples treated with Dissociation Reagent and diluted with Assay Buffer, one with a low diluted corticosterone level of 67.4 pg/mL and one with a higher diluted level of 658.0 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 Plasma	High Plasma	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	183.1	185.5	98.7
60%	40%	288.6	303.7	95.0
40%	60%	453.0	421.8	107.4
20%	80%	581.6	539.9	107.7
			<b>Mean Recovery</b>	<b>102.2%</b>

**Linearity**



**Intra Assay Precision**

Three mouse samples were diluted with 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	819.7	6.6
2	207.7	5.9
3	64.37	11.0

**Inter Assay Precision**

Three mouse samples were diluted with Assay Buffer and run in duplicates in fifteen assays run over multiple days by three operators. The mean and precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Conc. (pg/mL)	%CV
1	818.9	9.4
2	199.6	11.3
3	55.6	15.1

**SAMPLE VALUES**

Six random mammalian serum and plasma samples were tested in the EIA assay which uses identical antibody, conjugate and assay buffer. Neat sample values ranged from 0.87 to 38.5 µg/dL with an average for the human samples of 1.56 µg/dL. The normal reference range for serum corticosterone is 0.13-2.3 µg/dL.

Dried fecal samples were processed and run in the EIA assay. Samples kindly donated by Dr. J. Williams at the Indianapolis Zoo, which included Amur Tiger, Giraffe, Kudu, Lion, Reeves Muntjac, White Handed Gibbon, White Rhino, and Zebra, were tested and corticosterone values obtained ranged from 7.85 to 81.6 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered glucocorticoids are excreted in differing amounts in urine and feces across species, with fecal excretion ranging from 7% of administered cortisol in the pig to 82% in the cat. Palme has also shown that the peak of fecal glucocorticoid concentrations occur at 12 hours for sheep, but takes 48 hours to peak in pigs. It is therefore necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

**CROSS REACTIVITY**

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

Steroid	Cross Reactivity (%)
Corticosterone	100%
Desoxycorticosterone	12.30%
Tetrahydrocorticosterone	0.76%
Aldosterone	0.62%
Cortisol	0.38%
Progesterone	0.24%
Corticosterone-21-Hemisuccinate	<0.1%
Cortisone	< 0.08%
Estradiol	< 0.08%

**FOR RESEARCH USE ONLY**

**KAMIYA BIOMEDICAL COMPANY**

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