Corticosterone

CLIA kit

(96 Tests)

ZELLX GmbH (Germany)

CAT No. ZX-66100-96

www.zellx.de

Sample Types Validated for:

Serum, EDTA and Heparin Plasma, Dried Fecal Extracts, and Tissue Culture Media

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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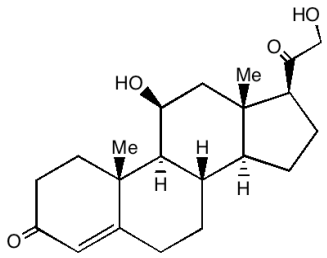
Please read this insert completely prior to using the product.

Introduction

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 secreted in response to stimulation of the adrenal cortex by Adrenocorticotropic hormone (ACTH), and is the precursor of aldosterone. Corticosterone is a major indicator of stress in non-human mammals. Studies have shown a link between corticosterone-mediated increased level of stress and impairment of long term memory retrieval, as well as chronic corticosterone elevation due to dietary restrictions and in response to burn injuries. In addition to stress levels, corticosterone is assumed to play a decisive role in sleep-wake patterns.



Assay principle

The ZellX® Corticosterone Immunoassay kit is designed to quantitatively measure Corticosterone present in serum, plasma, urine, extracted dried fecal samples, and tissue culture media samples. A corticosterone stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The assay standards range from either 78.125 to 5,000 to pg/mL or from 78.125 to 10,000 pg/mL. Please choose the standard range that fits your sample concentrations most appropriately.

The kit includes a 96-well plate that is pre-coated with a secondary donkey anti-sheep antibody. The function of this antibody is to capture the sheep anti-corticosterone antibody bound to corticosterone conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The corticosterone-conjugate (labeled) and the sample corticosterone (unlabeled) compete for binding to the sheep antibody. After 2 hours of incubation, the chemiluminescent substrate is added to react with the peroxidase-labeled antibody-antigen conjugate to produce light. The generated light can be measured in a microtiter plate reader capable of reading luminescence. The lower the amount of corticosterone in the sample, the stronger the signal due to more labeled corticosterone bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
Corticosterone Standard (50 ng/mL)	125 µL
Corticosterone CLIA Antibody	2.6 mL
Corticosterone CLIA Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
Dissociation Reagent	1 mL
Substrate A	5.6 mL
Substrate B	5.6 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

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

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate reader capable of reading glow chemiluminescence.

Note: All luminometers read Relative Light Units (RLU). These RLU readings will vary with brand or model of plate reader. The number of RLUs obtained depends on the sensitivity and gain of the reader used. If you are not sure how to properly configure your reader, contact your plate reader manufacturer or carry out the following protocol:

Dilute 5 µL of the Corticosterone Conjugate into 995 µL of deionized water. Pipet 5 µL of diluted conjugate into a white well and add 100 µL of prepared CLIA substrate. This well will give 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.

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

For Dried Fecal Sample:

ACS Grade Ethanol

Glass test tubes

Precautions

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.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- 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 Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by 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.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

iii. **Chemiluminescent Substrate:** Mix one part of the Substrate A with one part of Substrate B in a brown bottle. Chemiluminescent Substrate can be stored at 4°C for up to 1 month.

Sample preparation

Since Corticosterone is identical across all species, it is expected that this kit can measure corticosterone in human and other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

Dissociation Reagent must be used only with Serum and Plasma samples.

All samples and standards must be used within 2 hours of preparation or must be stored at ≤ -20 for later analysis.

I. **Serum, Plasma:**

- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Separate the serum or plasma from the red blood cells, and transfer into fresh tubes.
- Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Adding this reagent will yield the total corticosterone concentration in serum or plasma.
- Allow the Dissociation Reagent to warm completely to Room Temperature before use.
- Add 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 at least 5 minutes.
- Add 490 µL of Assay Buffer to the tube.
- This 1:100 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100.

II. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.

- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

III. **Tissue Culture Media:**

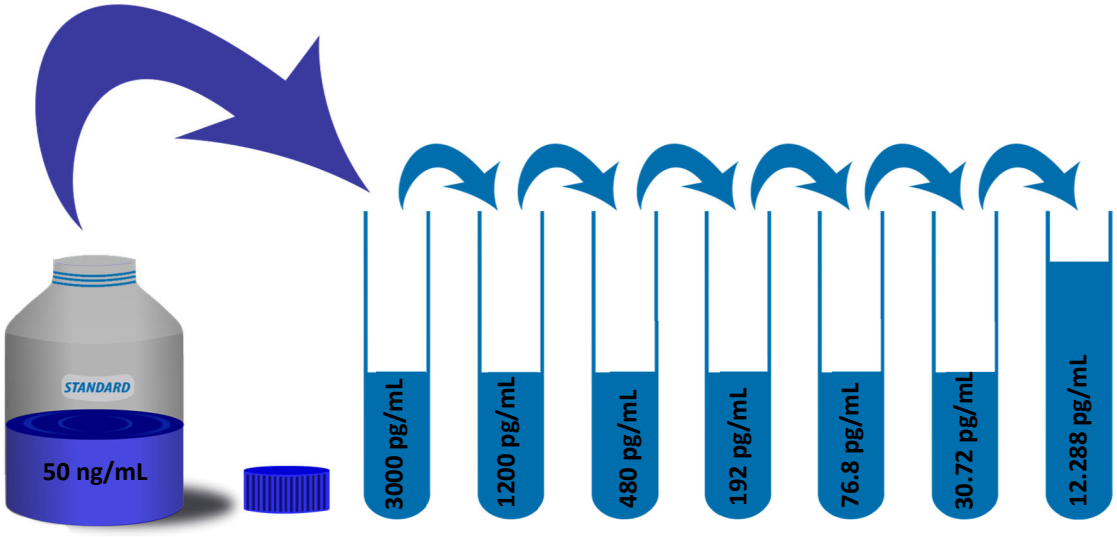
- For measuring corticosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

All the samples must be used within 2 hours of preparation; otherwise, aliquots of the sample should be kept at ≤ -20˚C for later use.

Standard preparation

- Prepare a 3:50 dilution of corticosterone Standard with Assay Buffer (mix 30 µL of standard with 470 µL of Assay Buffer), and label as the Standard No.7 (3000 pg/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.7	3000 pg/mL	30 µL corticosterone Standard + 470 µL Assay Buffer
Standard No.6	1200 pg/mL	150 µL Standard No.7 + 225 µL Assay Buffer
Standard No.5	480 pg/mL	150 µL Standard No.6 + 225 µL Assay Buffer
Standard No.4	192 pg/mL	150 µL Standard No.5 + 225 µL Assay Buffer
Standard No.3	76.8 pg/mL	150 µL Standard No.4 + 225 µL Assay Buffer
Standard No.2	30.72 pg/mL	150 µL Standard No.3 + 225 µL Assay Buffer
Standard No.1	12.288 pg/mL	150 µL Standard No.2 + 225 µL Assay Buffer
Standard No.0	0 pg/mL	225 µL Assay Buffer



All standard must be used within 2 hours of preparation

Assay Procedure

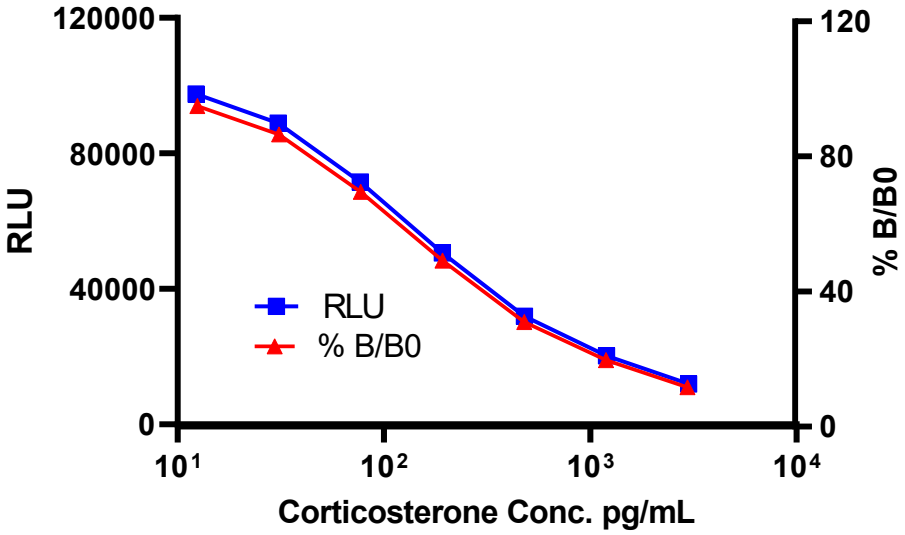
1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 75 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Corticosterone CLIA Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Corticosterone CLIA Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 2 hours at room temperature. If the plate is not shaken, signals will be approximately 45 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of Chemiluminescent Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 5 minutes without shaking.
12. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well.

➢ The chemiluminescent signal will decrease about 40% over 60 minutes.

Calculation

- Average the duplicate RLU readings for each standard and sample.
- Subtract the mean RLUs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

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



A typical standard curve of ZellX® Corticosterone CLIA Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® Corticosterone CLIA assay was determined as 12.8 pg/mL.

Sensitivity

The sensitivity of the ZellX® Corticosterone CLIA assay was determined as 6.71 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 mouse samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 mouse samples were tested in duplicate on 15 different assays over multiple days.

Item	*%CV*
Intra assay	6.6, 11.0, 5.9
Inter assay	9.4, 15.1, 11.3

Cross Reactivity

The following cross reactants were tested in the 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%

References

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