Corticosterone Competitive ELISA

Catalog Number	Kit Size
TBS31002-01	1x 96-well Plate
TBS31002-05	5x 96-well Plate

INTRODUCTION

Corticosterone 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. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns.

Tribioscience's Corticosterone Competitive ELISA employs the competitive sandwich enzyme immunoassay technique to detect corticosterone concentrations in a variety of samples from plasmid, serum and cell culture medium.

KIT CONTENT AND STORAGE CONDITIONS

PART#		Catalog Number	
		TBS31002-01	TBS31002-05
Corticosterone Microplate	TBS31002A	1x 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody.	5x 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody.
Corticosterone Standard	TBS31002B	1x 0.1mL: Corticosterone Standard (10 ng/mL).	5x 0.1mL: Corticosterone Standard (10 ng/mL).
Detection A	TBS31002C	1x 3mL: A corticosterone-peroxidase conjugate in a special stabilizing solution.	5x 3mL: A corticosterone-peroxidase conjugate in a special stabilizing solution.
Detection B	TBS31002D	1x 3mL: A polyclonal antibody specific for corticosterone.	5x 3mL: A polyclonal antibody specific for corticosterone.
Assay Diluent	TBS31002E	1x 2mL: 5x concentrate Assay Diluent.	1x 10mL: 5x concentrate Assay Diluent.
10x Wash Buffer	TBS3000W	1x 10mL: 10x concentrated solution.	1x 50mL: 10x concentrated solution.
TMB Substrate	TBS3000T	1x 12mL: ultra-sensitive TMB substrate.	5x 12mL: ultra-sensitive TMB substrate.
Stop Solution	TBS3000S	1x 6ml: 2N sulfuric acid.	1x 30 mL: 2N sulfuric acid.

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.

Other materials required, but provided in the Kit

Distilled or deionized water.
Repeater pipette with disposable tips capable of dispensing 25, 50, and 100µL.
Colorimetric 96-well microplate reader capable of reading optical density at 450nm.
Distilled or deionized water.
Ethyl acetate or ethanol for serum, plasma or fecal extracts.
A speedvac for evaporation of ethanol or ethyl acetate
A microplate shaker.
Colorimetric 96-well microplate reader capable of reading optical density at 450nm.
Software for converting raw relative optical density readings from the plate reader and carrying out four parameters logistic curve (4PLC) fitting.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

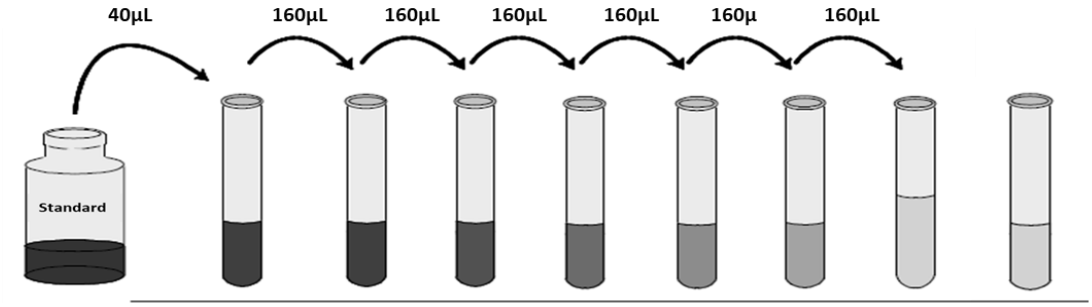
Bring all reagents to room temperature before use (around 30 mins).

1x Wash Buffer: Add 10mL of 10xWash Buffer Concentrate to 90mL of deionized water to prepare 100mL of 1xWash Buffer.

1x Assay Diluent: Dilute 2mL 5x Assay Buffer Concentrate 1:5 by adding 8ml deionized distilled water.

Standard Preparation

1. Label test tubes #1 through #7. Pipet 450µL of 1x Assay Buffer into tube #1 and 250µL into tubes #2 to #6 **as shown in the diagram below.**
2. Carefully add 50µL of the corticosterone stock solution to tube #1 and vortex completely *(Note: The corticosterone stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery).*



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	240	240	240	240	240	240	240
Stock (10 ng/mL)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Higher Std Vol. (µL)	40	160	160	160	160	160	160	
Final Conc (pg/mL)	10,000	4,000	1,600	640	256	102.4	40.96	

3. Take 250 µL of the corticosterone standard from tube #1 and add it to tube #2 and vortex completely. Repeat 2.5x serial dilutions for tubes #3 through #6. The concentration of corticosterone in tubes 1 through 6 will be 10,000, 4,000, 1,600, 256, 102.4 and 40.96 pg/mL. Use all Standards within 2 hours of preparation. Tube # 7 is Standard 0.

SAMPLE PREPARATION

Serum and Plasma Samples

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

Dilute above the sample with 490µL of 1x Assay Buffer. This 1:100 dilution can be diluted further with diluted Assay Buffer. Final serum and plasma dilution factor should be ≥ 1:100.

ASSAYPROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Use the plate layout sheet to aid in proper sample and standard 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. Pipette 50µL of samples or standards into wells in the plate.
3. Pipette 75µL of 1x Assay Buffer into the non-specific binding (NSB) wells.
4. Pipette 50µL of 1x Assay Buffer into the maximum binding (B0 or Zero standard) wells.

5. Add 25µL of Detection A (Corticosterone Conjugate) to each well using a repeater pipet.
6. Add 25µL of Detection B (Corticosterone Antibody) to each well, except the NSB wells, using a repeater pipette.
7. Gently tap the sides of the plate to ensure fully mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours (*Note: If the plate is not shaken signals bound will be approximately 45% lower*).
8. Aspirate the plate and wash each well 4x with 300µL wash buffer, 3 min each time. Tap the plate dry on clean absorbent towels.
9. Add 100µL of the TMB Substrate to each well, using a repeater pipette.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
12. Read the optical density (OD) in a microplate reader at 450nm.
13. 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 OF RESULTS

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 from 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

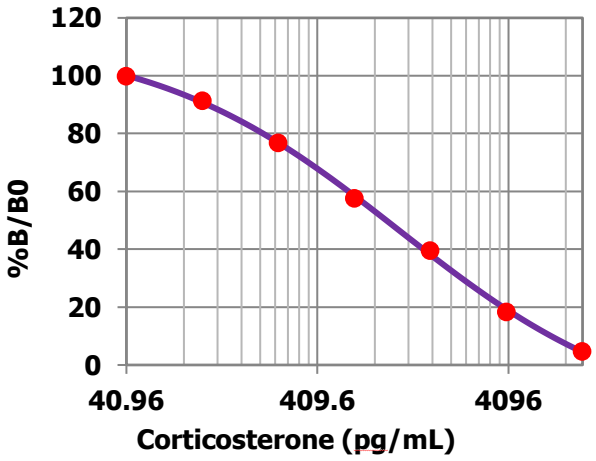


Table 1 TYPICAL DATA FOR 2-HOUR PROTOCOL

Sample	Mean OD	Net OD	% B/B0	Corticosterone conc. (pg/ml)
NSB	0.060	0		NSB
Standard 1	0.141	0.081	4.75	10000
Standard 2	0.372	0.312	18.23	4000
Standard 3	0.735	0.676	39.51	1600
Standard 4	1.044	0.984	57.54	640
Standard 5	1.371	1.311	76.68	256
Standard 6	1.621	1.561	91.31	102.4
Standard 7	1.767	1.707	99.84	40.96
B0	1.770	1.710	100	B0

Note: 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.

SENSITIVITY

Limit of Detection (LOD): The LOD for the assay is determine by the mean of OD from 6 wells run for each of the B0 minus two (2) standard deviations (SD). The LOD was 16.86 pg/mL in the assay.

SPECIFICITY

This assay recognizes natural and recombinant corticosterone 100% displayed as Table2.

RECOVERY

The corticosterone recovery spiked to levels throughout the range of the assay in various matrices listed as Table 3.

Table 2. Specificity of the Assay

Steroid	Cross Reactivity (%)
Corticosterone	100%
Tetrahydrocorticosterone	0.76%
Aldosterone	0.62%
Cortisol	0.38%
Progesterone	0.24%
Aldosterone	0.62%
Dexamethasone	0.12%
Corticosterone-21-hemisuccinate	< 0.1%
Cortisone	< 0.08%
Estradiol	< 0.08%

Table 3 Recovery in samples

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	94	89-99%
Serum (n=4)	92	86-112%
EDTA plasma (n=4)	91	87-115%
Heparin plasma (n=4)	89	79-107%
Urine (n=4)	101	95-106%

VALIDATION DATA

Intra Assay Precision

Samples were assayed in duplicates in 16 runs by four operators to determine precision between assays in Table 4.

Inter Assay Precision

Samples were assayed in replicates of 20 to determine precision within assay in Table 5.

Table 4 Intra-assay precision

Parameters	Sample 1	Samples 2	Samples 3
Mean (pg/mL)	1018.7	156.2	40.6
%CV	6	5.9	8.8

CV = Coefficient of Variation

Table 5 Inter-assay precision

Parameters	Sample 1	Samples 2	Samples 3
Mean (pg/mL)	1051.9	150.2	39.6
%CV	20.5	12.2	25.8

CV = Coefficient of Variation

REFERENCES

1. Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF. "Dietary restrictions causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks." J. Comp. Physiol, 2001; 171: 701-709.
2. Thellin O, Noel G, Khuana S, Ogle CK and Horseman ND "Stress hormone secretion and gut signal transducer (STAT) proteins after burn injury in rats." Shock, 2001; 16(5): 393-397.
3. Vazquez-Palacios G, et al, "Further definition of the effect of corticosterone on the sleep-wake pattern in the male rat." Pharmacol. Biochem Behavior, 2001: 70(2-3): 305-310.

RELATIVE PRODUCTS

Aldosterone Competitive ELISA (Catalog# TBS31001)

Human CEA ELISA (Catalog# TBS3210)

Tribo™ Human AFP ELISA (Catalog# TBS3212)

Tribo™ Human HE4 ELISA (Catalog# TBS3213)

Human IFN-Gamma ELISA Maxi (Catalog# TBS3230)

Protein Cell Lysis Buffer (Catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

TMB Substrate System (Catalog# TBS5021)

For research use only. Not for use in diagnostic procedures.

96-well Plate layout sheet:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												