Corticosterone Competitive ELISA Kit

Catalog Number EIACORT (96 tests), EIACORTX10 (10 x 96 tests)

Rev. 3.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Corticosterone ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of corticosterone in serum, EDTA and heparin plasma, urine, dried fecal extracts, and tissue culture media. The assay recognizes corticosterone independent of species.

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.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity (96 tests)	Quantity (10 x 96 tests)
Corticosterone Standard; 100,000 pg/mL corticosterone in a special stabilizing solution	125 µL	10 x 125 µL
Assay Buffer Concentrate (5X)	28 mL	10 x 28 mL
Antibody Coated Wells, 96-well strip-well plate coated with donkey anti-sheep IgG	1 plate	10 plates
Corticosterone Antibody	3 mL	10 x 3 mL
Corticosterone Conjugate	3 mL	10 x 3 mL
Dissociation Reagent	1 mL	10 x 1 mL
Wash Buffer Concentrate (20X)	30 mL	2 x 125 mL
TMB (Tetramethylbenzidine) Substrate	11 mL	10 x 11 mL
Stop Solution; contains 1 M HCl, CAUSTIC	5 mL	1 x 50 mL
Plate Sealer	1	10

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Plate shaker
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge, or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

Sample type	Procedure
Serum and plasma	1. Warm Dissociation Reagent to room temperature. 2. Add 5 µL warm Dissociation Reagent into a microcentrifuge tube. 3. Add 5 µL of sample to the microcentrifuge tube. 4. Vortex gently and incubate at room temperature for at least 5 minutes. 5. Add 490 µL of 1X Assay Buffer to prepare a 1:100 dilution of serum or plasma sample. Dilute further if necessary with 1X Assay Buffer to perform the assay. Final serum and plasma dilutions should be ≥ 1:100.
Urine	Dilute samples ≥1:20 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. No. EIACUN) is available for measuring urine creatinine for normalization of corticosterone levels in a random urine specimens.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com** **Note**: The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be <5%.
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

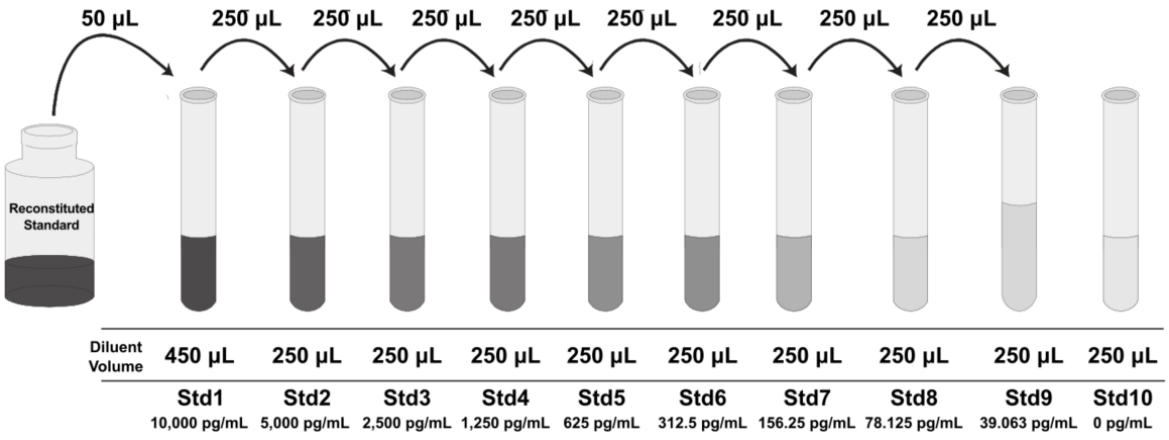
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 10,000 to 39.063 pg/mL, but a curve can be obtained using a range of 5,000 to 39.063 pg/mL. Choose the range that fits your sample concentrations most appropriately.

The Corticosterone Standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.

1. Add 50 µL Corticosterone Standard to one tube containing 450 µL 1X Assay Buffer and label as 10,000 pg/mL corticosterone.
2. Add 250 µL 1X Assay Buffer to each of 8 tubes labeled as follows: 5,000; 2,500; 1,250; 625; 312.5; 156.25; 78.125; 39.063; and 0 pg/mL corticosterone.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
   Use the standards within 2 hours of preparation.

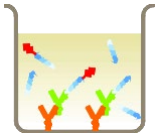


Perform ELISA (Total assay time: 1.5 hours)

IMPORTANT! Perform a standard curve with each assay.

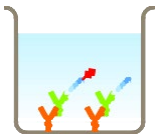
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



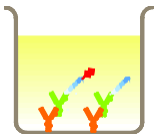
Bind antigen

a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of Corticosterone Conjugate to each well.
d. Add 25 µL of Corticosterone Antibody to each well except NSB wells.
e. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 1 hour at room temperature with shaking.
   Note: If the plate is not shaken the bound of the signals will be ~45% lower.
f. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.
   Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns, and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–10,000 pg/mL corticosterone.

Standard Corticosterone (pg/mL)	Optical Density (450 nm)*
10,000	0.222
5,000	0.297
2,500	0.398
1,250	0.550
625	0.719
312.5	0.926
156.25	1.049
78.125	1.151
39.063	1.220
0	1.291

Note: The NSB gave a Mean OD value of 0.087.

Intra-assay precision

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

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

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicate 14 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	2,618.3	630.1	267.9
%CV	7.5	6.4	9.9

CV = Coefficient of Variation

Expected values

Random mammalian serum and plasma samples (n=6) and dried fecal samples were tested in the assay.

Sample	Range	Average
Serum/plasma (neat)	0.87–38.5 µg/dL	1.56 µg/dL [1]
Dried fecal material [2]	7.85–81.6 pg/mg	—

[1] Value for human samples. The normal reference range for serum corticosterone is 0.13–2.3 µg/dL.

[2] Samples from Amur Tiger, Giraffe, Kudu, Lion, Reeves Muntjac, White Handed Gibbon, White Rhino, and Zebra.

Analysis of radiolabeled glucocorticoids indicate that administered glucocorticoids are excreted in differing amounts across species, and that the peak of glucocorticoid concentrations can vary. It is therefore necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

Recovery

Recovery was determined by assaying high and low concentration serum samples treated with Dissociation Reagent and diluted 1:50 with Assay Buffer (high sample 2,890.5 pg/mL corticosterone; low sample 104.6 pg/mL corticosterone) mixed in the ratios shown in the following table.

Low Sample %	HIgh Sample %	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%

Specificity

The following samples were tested using the assay, with cross-reactivity 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
Dexamethasone	0.12
Corticosterone-21-Hemisuccinate	<0.1
Cortisone	<0.08
Estradiol	<0.08

Sensitivity

The analytical sensitivity of the assay is 20.9 pg/mL corticosterone. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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23 February 2024