Cortisol Competitive ELISA Kit

Catalog Number EIAHCOR (96 tests), EIAHCORX10 (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 Cortisol Competitive ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of cortisol in serum, plasma (EDTA and heparin), dried fecal extracts, urine, saliva, or tissue culture medium. Total cortisol is measured in extracted samples, serum, and plasma; while free cortisol is measured in urine, and saliva.

The assay was validated with samples from human, but is expected to measure cortisol in samples from other species.

Cortisol ($C_{21}H_{30}O_5$, hydrocortisone, Kendall's Compound F) is a glucocorticoid secreted by the adrenal cortex. It is produced in response to stress and affects blood pressure, blood sugar levels, and other functions of stress adaptation. Production of cortisol follows an ACTH-dependent circadian rhythm, with peak levels in the morning and decreasing levels throughout the day. Most serum cortisol (96%) is bound to proteins including corticosteroid binding globulin and serum albumin.

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)
Cortisol Standard; 32,000 pg/mL cortisol in a special stabilizing solution	125 µL	10 x 125 µL
Assay Buffer Concentrate (5X)	28 mL	10 x 28 mL
Clear 96-well Plate, 96-well strip-well plate; goat anti-mouse IgG coated	1 plate	10 plates
Cortisol Antibody	3 mL	10 x 3 mL
Cortisol 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
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- 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.

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.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- 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.

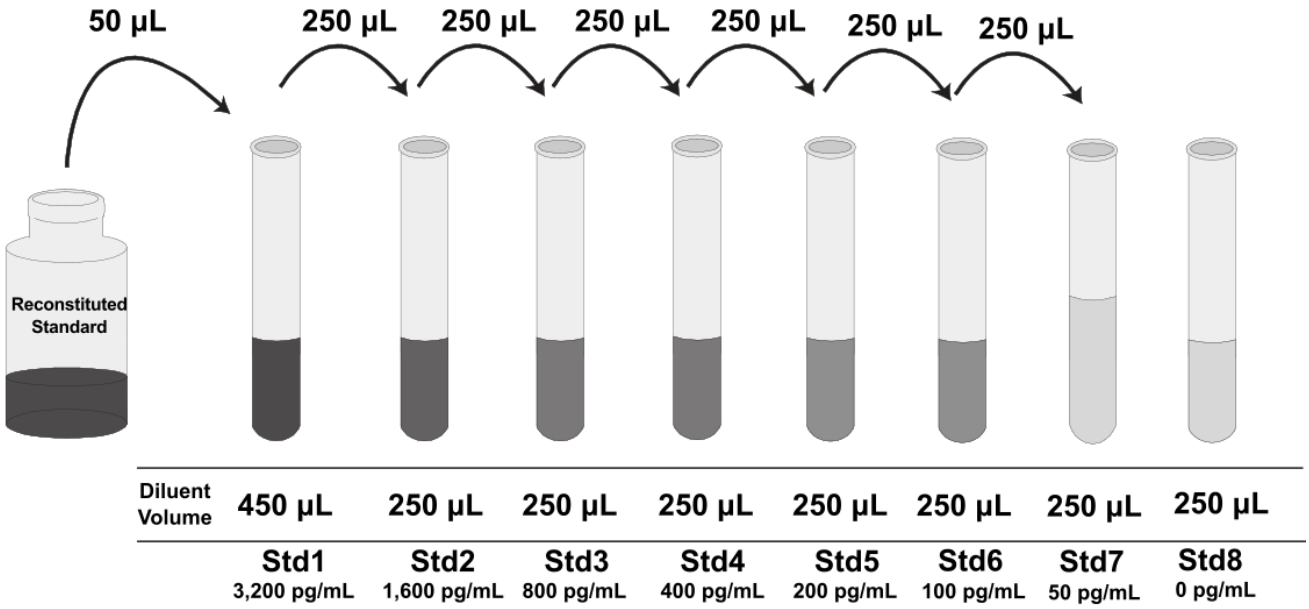
Sample type	Procedure
Serum and plasma	1. Warm Dissociation Reagent to room temperature. 2. Add 5 µL warm Dissociation Reagent into a microcentrifuge tube. 3. Add 5 µL of sample to the microcentrifuge tube. 4. Vortex gently and incubate at room temperature for at least 5 minutes. 5. Add 490 µL of 1X Assay Buffer to prepare a 1:100 dilution of serum or plasma sample. Dilute further with 1X Assay Buffer to perform the assay. Final serum and plasma dilutions should be ≥1:100.
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%.
Urine	Dilute samples ≥1:8 with 1X Assay Buffer. **Note**: A Creatinine Urinary Detection Kit (Cat. No. EIACUN) is available for measuring urine creatinine for normalization of corticosterone levels in a random urine specimens.
Saliva	Dilute samples ≥1:4 with 1X Assay Buffer. See detailed handling procedures on the product page at **thermofisher.com**
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Important: The Cortisol 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 dispensed accurately.

1. Add 50 µL Cortisol Standard to one tube containing 450 µL 1X Assay Buffer and label as 3,200 pg/mL cortisol.
2. Add 250 µL 1X Assay Buffer to each of 7 tubes labeled as follows: 1,600; 800; 400; 200; 100; 50; and 0 pg/mL cortisol.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

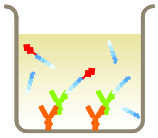


Perform ELISA (Total assay time: 1.5 hours)

IMPORTANT! Perform a standard curve with each assay.

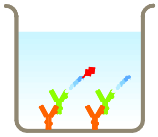
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

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.



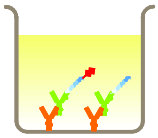
Bind antigen

a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of Cortisol Conjugate to each well.
d. Add 25 µL of Cortisol 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.
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.
   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 the appropriate diluent 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 0–3,200 pg/mL cortisol.

Standard Cortisol (pg/mL)	Optical Density (450 nm)
3,200	0.129
1,600	0.200
800	0.324
400	0.458
200	0.575
100	0.646
50	0.681
0	0.778

Note: The NSB gave a Mean OD value of 0.080.

Intra-assay precision

Samples were assayed 20 times to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,174.3	475.9	177.4
%CV	6.0	5.6	14.7

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 10 times in duplicate by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,188.1	508.7	199.7
%CV	7.2	6.3	10.9

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Random mammalian samples were evaluated for the presence of cortisol in this assay.

Sample	Range	Average
Serum/plasma (n=6) [1]	8.5–23.8 µg/dL	12.2 µg/dL
Urine (n=4)	98.1–304.9 µg/g [2]	159.8 µg/g [2]
Dried fecal material [3]	2.48–27.22 pg/mg	—

[1] Value for human samples. The normal reference range for serum cortisol is 3–23 µg/dL (30–230 ng/mL).

[2] Values determined with the Creatinine Urinary Assay Kit (Cat. No. EIACUN), and normalized in µg/g creatinine. The normal reference range for urinary cortisol is 0.7–119 µg/g of creatinine (approximately 100,000 to 1,000,000 pg/mL in 24 hour urine samples).

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

Linearity of dilution

Linearity was determined by assaying high and low concentration human urine samples (high sample 2,974.9 pg/mL; low sample 163.9 pg/mL) mixed in the ratios shown in the following table.

Low Sample %	High Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% Recovery
80	20	726.1	715.7	98.6
60	40	1,288.3	1,311.5	101.8
40	60	1,850.5	1,683.3	91.0
20	80	2,412.7	2,306.3	95.6
			Mean Recovery	**96.7%**

Specificity

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

Sample	Cross reactivity (%)
Cortisol	100
Dexamethasone	18.8
Prednisolone (1-dehydrocortisol)	7.8
Corticosterone	1.2
Cortisone	1.2
Progesterone	<0.1
Estradiol	<0.1
Cortisol 21-glucuronide	<0.1

Sensitivity

The analytical sensitivity of the assay is 27.6 pg/mL cortisol. 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

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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