# Cortisone Competitive ELISA Kit

**Catalog Number** EIACOR (96 tests), EIACORX10 (10 x 96 tests)

**Rev** 2.0

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 Cortisone Enzyme Immunoassay is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of cortisone in dried fecal extracts, urine, saliva, plasma, and serum. The assay recognizes cortisone independent of species.

## 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) |
|---|---|---|
| Cortisone Standard; 1,000 ng/mL cortisone 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 goat anti-rabbit IgG | 1 plate | 10 plates |
| Cortisone Antibody | 3 mL | 10 x 3 mL |
| Cortisone 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 shaker
- 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

**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.

## 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

- 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.<br>2. Add 5 µL warm Dissociation Reagent into a microcentrifuge tube.<br>3. Add 5 µL of sample to the microcentrifuge tube.<br>4. Vortex gently and incubate at room temperature for at least 5 minutes.<br>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. |
| Urine | Dilute samples >1:5 with 1X Assay Buffer. Due to levels found in urine, dilutions may need to be >1:100.<br>**Note**: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for measuring urine creatinine for normalization of cortisone in a random urine specimens. |
| Saliva | Saliva samples should be collected in a Saliva Collection Device or frozen and thawed, then centrifuged at 14,000 rpm for 15 minutes. The supernatant should be diluted ≥ 1:5 with 1X Assay Buffer prior to running the assay. |
| Dried feces | See detailed extraction protocol on the product page at **thermofisher.com**<br>**Note**: Ethanol concentration in the final diluted Assay Buffer dilution added to the well should be <2.5%. |
| Tissue culture media | Perform sample dilutions with the corresponding tissue culture medium. |

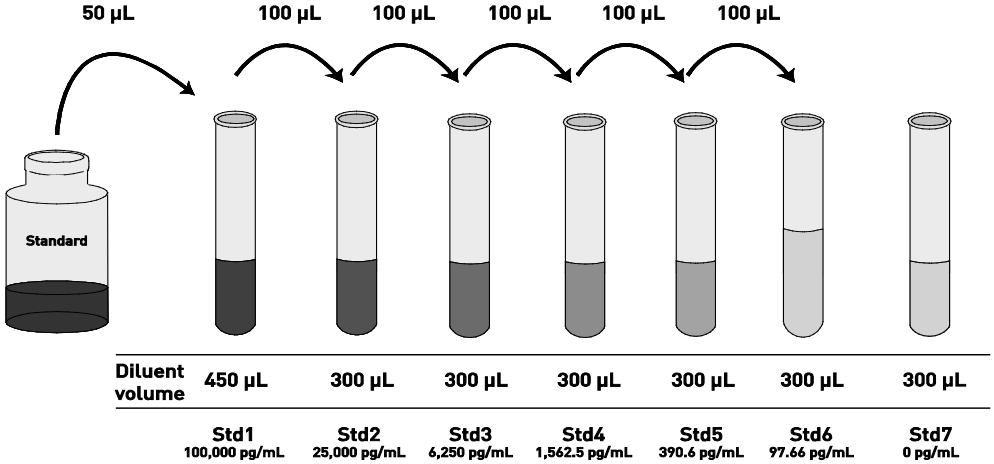
## Dilute standards

**Note:** Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 100,000 to 97.66 pg/mL, but a curve can be obtained using a range of 25,000 to 97.66 pg/mL. Choose the range that fits your sample concentrations most appropriately.

The Cortisone 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 Cortisone Standard to one tube containing 450 µL 1X Assay Buffer and label as 100,000 pg/mL cortisone.
2. Add 300 µL 1X Assay Buffer to each of 7 tubes labeled as follows: 100,000; 25,000; 6,250; 1,562.5; 390.6; 97.66; and 0 pg/mL cortisone.
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**.

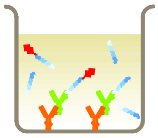


## Perform ELISA (Total assay time: 2.5 hours)

**IMPORTANT!** Perform a standard curve with each assay.

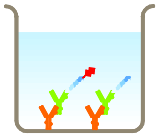
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

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.



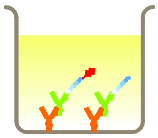
### Bind antigen

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 Cortisone Conjugate to each well.
d. Add 25 µL of Cortisone 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 2 hours at room temperature with shaking.
   **Note**: If the plate is not shaken the bound of the signals will be ~20% 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 0–100,000 pg/mL cortisone.

| Standard Cortisone (pg/mL) | Optical Density (450 nm)* |
|---|---|
| 100,000 | 0.243 |
| 25,000 | 0.372 |
| 6,250 | 0.540 |
| 1,562.5 | 0.746 |
| 390.6 | 0.966 |
| 97.66 | 1.167 |
| 0 | 1.326 |

**Note:** The NSB gave a Mean OD value of 0.066.

### Intra-assay precision

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 14,123 | 2,105 | 349.2 |
| %CV | 7.4 | 12.1 | 6.2 |

CV = Coefficient of Variation

### Inter-assay precision

Samples were assayed in duplicates in 19 assay runs by multiple operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 12,165 | 1,976 | 366.1 |
| %CV | 12.7 | 10.2 | 12.9 |

CV = Coefficient of Variation

## Performance characteristics, continued

### Expected values

Eight human serum and six human plasma samples were tested in the assay. Neat sample values ranged from 18.5 to 62.2 ng/mL with an average of 38.6 ng/mL. Two normal human saliva samples were tested in the assay and read between 6.0 ng/mL and 12.8 ng/mL. Nine normal human urine samples were also tested. The samples read from 78.4 to 344.8 ng/mL with an average of 203.6 ng/mL.

Dried fecal samples were processed and run in the assay. Cortisone values obtained ranged from 1.1 to 62 ng/100 mg dried fecal material. It has been shown that radiolabeled, administered glucocorticoids are excreted in differing amounts in urine and feces across species, with fecal excretion ranging from 7% of administered glucocorticoid in the pig to 82% in the cat. Researchers have also shown that the peak of fecal concentrations occurs at 12 hours for sheep, but takes 48 hours for pigs. It is necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

### Recovery

Recovery was determined in urine and serum samples diluted with Assay Buffer by mixing samples with high and low levels of cortisone in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

| High Sample % | Low Sample % | Expected Conc. (pg/mL) | | Observed Conc. (pg/mL) | | % Recovery | |
|---|---|---|---|---|---|---|---|
| | | Serum | Urine | Serum | Urine | Serum | Urine |
| 80 | 20 | 310.7 | 21,373 | 338.3 | 22,447 | 108.9 | 105.0 |
| 60 | 40 | 253.7 | 16,274 | 302.8 | 17,263 | 119.4 | 106.1 |
| 40 | 60 | 196.7 | 11,175 | 223.7 | 11,676 | 113.8 | 104.5 |
| 20 | 80 | 139.7 | 6,075 | 130.9 | 6,405 | 93.7 | 105.4 |
| | | | | **Mean Recovery** | | **108.9%** | **105.3%** |

### Specificity

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

| Steroid | Cross-reactivity % |
|---|---|
| Cortisone | 100 |
| 5α-Dihydrocortisone | 31.7 |
| Prednisone | 9.0 |
| 5ß-Dihydrocortisone | 4.4 |
| 11-Dehydrocorticosterone | 0.62 |
| 20α-Dihydrocortisone | 0.26 |
| 1α-Hydroxycorticosterone | <0.1 |
| 20ß-Dihydrocortisone | <0.1 |
| Corticosterone | <0.1 |
| Cortisol | <0.1 |
| Dexamethasone | <0.1 |
| Estradiol | <0.1 |
| Progesterone | <0.1 |

### Sensitivity

The analytical sensitivity of cortisone is 29.0 pg/mL. 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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