BioOcean® Cortisol Competitive ELISA Kit

Catalog Number EK8100
Size: 96 Test

For the quantitative determination of cortisol concentrations in cell culture supernates, serum and plasma.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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QUICK PROCEDURE FLOWCHART

1. **Prepare all reagents and standards as directed.**

2. **Add 300 µl *Washing Buffer (1×)* per well to soak for about 30 seconds. Use immediately after aspirate.**

3. **Add 50 µl of diluted *Antibody* to each well except Blank, NSB and TA wells.**

4. **Incubate for 1.5 hours at RT. Aspirate and wash 6 times.**

5. **Add 100 µl of serial diluted *Standard* to Standard wells in duplicate. Add 100 µl of prepared samples to Sample wells. Add 100 µl of *Standard Diluent* to NSB and B0 wells. (The treatment refers to the Sample Preparation on Page 6).**

6. **Add 50 µl of diluted *Cortisol Conjugate* to each well except Blank and TA wells.**

7. **Incubate for 2 hours at RT. Aspirate and wash 6 times.**

8. **Add 5 µl of diluted *Cortisol Conjugate* to TA well.**

9. **Add 100 µl *Substrate Solution* to each well.**

Incubate for 5 - 30 minutes at RT. Protect from light.

10. **Add 100 µl *Stop Solution* to each well.**

11. **Read at 450 nm within 30 minutes. Correction 570 or 630 nm.**

DESCRIPTION

Cortisol, also known as hydrocortisone or compound F, is a steroid hormone, in the glucocorticoid class of hormones. It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to stress and low blood-glucose concentration, therefore, cortisol can be used as a biomarker of stress. Production of cortisol follows an ACTH-dependent circadian rhythm, with peak levels in the morning and decreasing levels throughout the day. Cortisol can be measured in many matrices including blood, feces, urine, and saliva. Most serum cortisol (90 - 95 %) is bound to proteins including corticosteroid binding globulin and serum albumin.

Cortisol is involved primarily in metabolic and immunological actions. In the metabolic aspect, it promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Immunologically, Cortisol functions as an important anti-inflammatory, and plays a role in hypersensitivity, immunosuppression, and disease resistance. Abnormal Cortisol levels are being tested for correlation with a variety of different conditions, these include: prostate cancer, depression, and schizophrenia. It is already known that an excess of Cortisol in all bodily tissues is the cause of Cushing's Syndrome.

PRINCIPLE OF THE ASSAY

BioOcean Cortisol Competitive ELISA Kit is based on the quantitative enzyme-linked immunosorbent assay technique to measure concentration of cortisol in the samples. Rabbit anti-mouse IgG polyclonal antibody has been pre-coated onto a microplate. Monoclonal antibody specific for cortisol is added into the wells, and is bound by the immobilized antibody following incubation. After washing, HRP-labeled cortisol and purified cortisol/sample is added to compete for limited sites on the monoclonal antibody. After washing away any unbound substances, substrate solution is added to the wells and color develops in inverse proportion to the amount of cortisol. The color development is stopped and the intensity of the color is measured. The assay is validated with serum samples from human, mouse and rat, but is expected to measure cortisol in samples from other species.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Buffer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- Bacterial or fungal contamination in either samples or reagents, or cross-contamination between reagents may cause erroneous results.

MATERIALS PROVIDED

Unopened kit should be stored at 2 - 8℃. 

- **Rabbit anti-mouse IgG Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse IgG.
- **Cortisol Antibody** (1 vial, 60 µl): 100× liquid.
- **Cortisol Standard** (1 vial, 150 µl): Recombinant cortisol in organic solvent; 10× liquid.
- **Cortisol Conjugate** (1 vial, 100 µl): Recombinant HRP-conjugated cortisol in a stabilizing solution; 100× liquid.
- **Standard Diluent** (1 bottle, 5 ml): In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
- **Dissociation Reagent** (1 bottle, 10 ml).
- **Assay Buffer (10×)** (1 bottle, 10 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (5 strips).

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8℃ (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Standard Diluent Substrate TMB Cortisol Antibody Cortisol Standard Cortisol Conjugate	Up to 1 month at 2 - 8℃.
	Standard	Up to 1 month at ≤ -20℃ in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
	Microplate Wells	Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- Intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Treat all chemicals with caution because they can be potentially hazardous.
- It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves.
- Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice.
- Pure water or deionized water must be used for reagent preparation.
- The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution.
- Do not expose kit reagents to strong light during storage and incubation.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
- In some cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at 6,000 × g for 5 minutes.

TECHNICAL HINTS

- All reagents including microplate, samples, standards and working solution should be warmed to room temperature before use.
- To obtain accurate results, using adhesive film to seal the plate during incubation is suggested.
- It is recommended that all samples and standards be assayed in duplicate.
- Avoid foaming when mixing or reconstituting solutions containing protein.
- To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips between each standard, sample and reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- When pipetting reagents, maintain a consistent order of addition from well-to-well.
- Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development.
- Read absorbance within 30 minutes after adding stop solution.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer maybe needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃ to avoid loss of bioactive cortisol. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

For serum samples, pretreatment should be performance to remove potentially interfering proteins and cortisol-binding protein.

1. Add 100 µl serum and 100 µl Dissociation Reagent to a microcentrifuge tube. Mix well.
2. Incubate for 15 minutes at room temperature. Centrifuge at ≥ 12,000 × g for 4 minutes.
3. Pipet 50 µl supernate carefully, and transfer to a new microcentrifuge tube, add 950 µl Assay Buffer (1×), mix well.

REAGENT PREPARATION

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1000 ml graduated cylinder.
Bring to final volume of 1000 ml with pure or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (10 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder.
Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Cortisol Antibody

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Cortisol Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Cortisol Antibody should be used within 30 minutes after dilution.

Cortisol Conjugate

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Cortisol Conjugate** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Cortisol Conjugate should be used within 30 minutes after dilution.

Sample Dilution

If your samples have high cortisol content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Cortisol Standard

The concentration of provided Cortisol Standard is 150 ng/ml. The Cortisol Standard contains an organic solvent. Pipette the Standard up and down several times to wet the pipet tip before transfer to insure that volumes are dispensed accurately.
Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated cortisol standard* (50 µl) with 450 µl of *Standard Diluent* creates the high standard (15,000 pg/ml). Pipette 250 µl of *Standard Diluent* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Standard Diluent* serves as the zero standard (0 pg/ml).

For cell culture supernates, mixing *concentrated cortisol standard* (50 µl) with 450 µl of cell culture medium without serum creates the high standard (15,000 pg/ml). Pipette 250 µl of cell culture medium without serum into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium without serum serves as the zero standard (0 pg/ml).

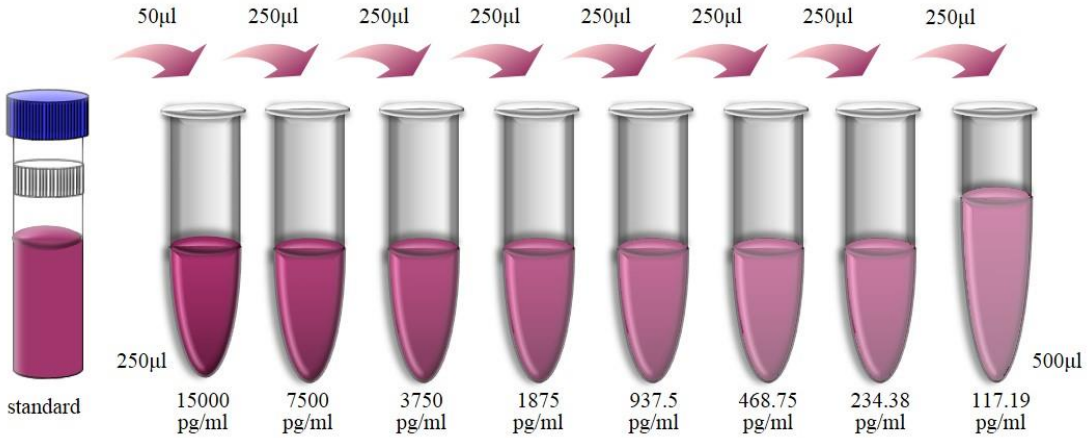


PLATE SETUP

Each plate or set of strips must contain a minimum of two Blanks, two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight point standard curve run in duplicate.

Note: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format and pipetting summary are shown below. The user may vary the location and type of wells present as necessary for each particular experiment.

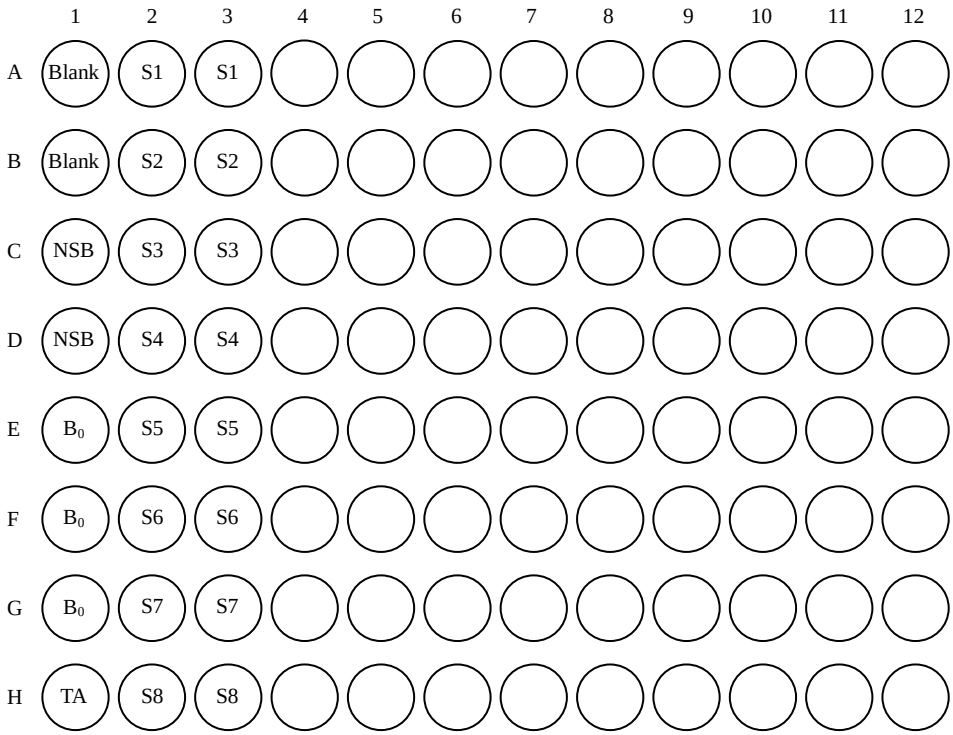
Component / Well	Standard Diluent	Antibody	Standard	Sample	Conjugate
Blank	-	-	-	-	-
NSB	100 µl	-	-	-	50 µl
B_0	100 µl	50 µl	-	-	50 µl
TA	-	-	-	-	5 µl (at develop step)
Standard	-	50 µl	100 µl	-	50 µl
Sample	-	50 µl	-	100 µl	50 µl

Blank: background absorbance.

NSB (Non-Specific Binding): non-immunological binding of the conjugate to the well.

TA (Total Activity): total enzymatic activity of the conjugate.

B_0 (Maximum Binding): maximum amount of the conjugate that the antibody can bind in the absence of free analyte.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 µl *Washing Buffer (1×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 50 µl of diluted *Antibody* to each well **except Blank, NSB and TA wells**.
5. Cover with an adhesive strip. Incubate at room temperature (18 to 25℃) for 1.5 hours on a microplate shaker set at 300 rpm.
6. Aspirate each well and wash by filling each well with 300 µl *Washing Buffer (1×)*, repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining *Washing Buffer (1×)* by aspirating or decanting. Invert the plate and tap it against clean paper towels.
7. Add 100 µl of serial diluted *Standard* to Standard wells in duplicate. Add 100 µl of prepared samples to Sample wells. Add 100 µl of *Standard Diluent* to NSB and B_0 wells. (The dilution refers to the Sample Preparation on Page 6).
8. Add 50 µl of diluted *Cortisol Conjugate* to each well **except Blank and TA wells**. Ensure reagent addition in step 7 and 8 is uninterrupted and completed within 15 minutes.
9. Cover with an adhesive strip. Incubate at room temperature (18 to 25℃) for 2 hours on a microplate shaker set at 300 rpm.

10. Repeat aspiration/wash as in step 6.
11. Add 5 µl of diluted *Cortisol Conjugate* to TA well.
12. Add 100 µl of *Substrate Solution* to each well. Incubate for 10 - 30 minutes at room temperature. Protect from light.
13. Add 100 µl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate readings for each standards (including B_0) and sample, and subtract the average NSB optical density (O.D.).

% B/B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

Plot % B/B_0 for standards S1 - S8 versus cortisol concentration using linear (y) and log (x) axes and draw the best-fit curve through the plotted points (e.g. 4-parameter logistic).

Calculate the concentration of cortisol corresponding to the mean absorbance from the standard curve.

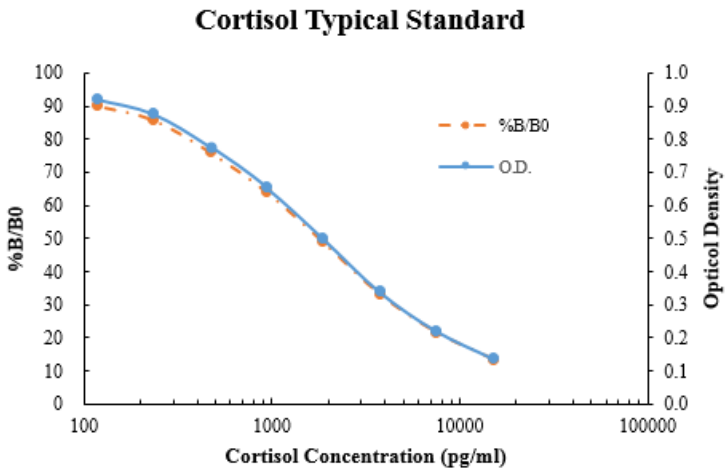
Note: Blank and TA values are not used in the standard curve calculations. Rather, they are used as diagnostic tools.

The finally concentration of top standard is 15,000 pg/ml. If samples have been diluted following the instruction, the final dilution factor is 40. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

TYPICAL DATA

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

(pg/ml)	O.D.		Average	Corrected	%B/B0
NSB	0.004	0.003	0.004	-	-
B0	1.021	1.029	1.025	1.021	-
117.19	0.911	0.938	0.925	0.921	90.2
234.38	0.867	0.894	0.881	0.877	85.9
468.75	0.765	0.799	0.782	0.778	76.2
937.50	0.660	0.658	0.659	0.655	64.2
1875.00	0.495	0.515	0.505	0.501	49.1
3750.00	0.334	0.353	0.344	0.340	33.3
7500.00	0.226	0.226	0.226	0.222	21.7
15000.00	0.148	0.138	0.143	0.139	13.6



SENSITIVITY

The minimum detectable dose (MDD) of cortisol is typically about 66.43 pg/ml.
The MDD was determined by subtracting two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)
Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision				Inter-assay precision		
Sample	1	2	3		1	2	3
n	20	20	20		6	6	6
Mean (pg/ml)	1146.5	3122.0	8249.1		872.4	2743.7	7585.6
Standard deviation	52.6	143.5	354.5		63.5	213.3	485.5
CV (%)	4.6	4.6	4.3		7.3	7.8	6.4

RECOVERY

The spike recovery was evaluated by spiking 3 levels of cortisol into five health serum samples. The un-spiked serum was used as blank in these experiments.
The recovery ranged from 80 % to 130 % with an overall mean recovery of 118 %.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of cortisol in serum and diluted with Standard Diluent to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	85	80 - 112
1:4	98	88 - 109
1:8	105	90 - 116
1:16	118	105 - 120

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant cortisol produced at BioOcean. The conversion formula is as follow: 1 pg/ml = 2.759 pmol/L.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers/mice/rats were evaluated for the presence of cortisol in this assay.

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Detectable (%)	Mean of Detectable (ng/ml)
Human serum	30	47.5 - 157.4	100	104.6
Mouse serum	30	18.2 - 24.1	100	21.1
Rat serum	30	11.4 - 31.2	100	23.4

Note: The sample range is non-physiological range. The sample range of healthy human/mice/rats will difference according to geographical, ethic, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

Compound	Cross-reactivity
Cortisol	100 %
Deoxycortisol	0.9 %
Prednisolone	5.6 %
Corticosterone	0.6 %
11-Deoxycorticosterone	< 0.1 %
Progesterone	< 0.1 %
17-Hydroxyprogesterone	< 0.1 %
Testosterone/Estradiol/Estriol	< 0.1 %
Danazol	< 0.01 %