FluoProbes

NIST-Calibrated Kit

Cortisol
Enzyme Immunoassay Kit

1 or 5 Strip Plates Catalog Number FP-1C3690 or FP-1C3692
1 or 5 Whole Plates Catalog Number FP-1C3691 or FP-1C3693

Species Independent

Sample Types Tested:

Dried Fecal Extracts, Saliva, Urine
Serum, EDTA and Heparin Plasma and
Tissue Culture Media

Please read this insert completely prior to using the product.

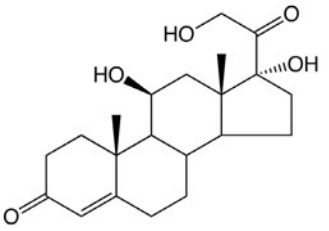
For research use only. Not for use in diagnostic procedures

Table of Contents

Background	3
Assay Principle	4
Related Products	4
Supplied Components	5
Storage Instructions	5
Other Materials Required	6
Precautions	6
Sample Types	7
Sample Preparation	7
Reagent Preparation	8
Assay Protocol	9
Calculation of Results	10
Typical Data	10-11
Validation Data Sensitivity, Linearity, etc.	11-13
Sample Values and Cross Reactivity	14
Warranty & Contact Information	15
Plate Layout Sheet	16

Background

Cortisol, $C_{21}H_{30}O_5$, (hydrocortisone, compound F) is the primary glucocorticoid produced and secreted by the adrenal cortex. It is often referred to as the "stress hormone" as it is involved in the response to stress and it affects blood pressure, blood sugar levels, and other actions of stress adaptation. Immunologically, cortisol functions as an important anti-inflammatory and plays a role in hypersensitivity, immunosuppression, and disease resistance[1]. In the metabolic aspect, cortisol promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization[2]. Production of cortisol follows an ACTH-dependent circadian rhythm, with a peak level in the morning and decreasing levels throughout the day. Most serum cortisol, all but about 4%, is bound to proteins including corticosteroid binding globulin and serum albumin[3]. Only free cortisol is available to most receptors and it is through these receptors that physiological processes are modulated. Abnormal cortisol levels are being evaluated for correlation with a variety of different conditions, such as prostate cancer[4], depression[5], and schizophrenia[6]. It is already known that abnormal levels of cortisol are involved in Cushing's Syndrome and Addision's disease[7].



1. E. Friess, et al., Eur J Clin Invest, 2000, 30, Suppl 3:46-50.
2. Freeman, Scott, 2002. Biological Science. Prentice Hall; 2nd Pkg edition (December 30, 2004).
3. C. Longscope., J. Endocrinology, 1996, , Suppl S125-S127.
4. J. Herbert, Lancet, 1995 345, 1193-1194.
5. A. Michael, et al., Biol. Psychiatry, 2000, 48, 989-95.
6. C.R. Dequet and D.J. Wallace, Current Opin. Ivest. Drugs, 2001, 8, 1045-53.
7. W.M. Jeffries, Med. Hypotheses, 1998, 51, 114-4.

Assay Principle

The FluoProbes Cortisol Immunoassay kit is designed to quantitatively measure cortisol present in dried fecal extracts, saliva, urine, serum, plasma and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total cortisol in extracted samples and in serum and plasma and free cortisol in saliva and urine. A cortisol standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture mouse antibodies. A cortisol-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to cortisol to each well. After an 1 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound cortisol-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the cortisol in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

Related Products

KITS

Urinary Creatinine Detection Kit (2 Plate)
Urinary Creatinine Detection Kit (10 Plate)
Cortisol Enzyme Immunoassay Kit (1 Plate)
Corticosterone Enzyme Immunoassay Kit
Corticosterone Enzyme Immunoassay Kit (5 Plate)

REAGENTS

Cortisol Affinity Resin
500 µL Resin with > 2.5 mg Sheep anti-Cortisol covalently bound

Supplied Components

Clear Coated 96 Well Plate
Each are coated with goat anti-mouse IgG.

Kit 1C3690 or 1C3692	1 or 5 Plate	Catalog Number 1C369a, 1 x 8 Strip Well
Kit 1C3691 or 1C3693	1 or 5 Plate	Catalog Number 1C369m, Whole Well

Cortisol Standard
Cortisol at 32,000 pg/mL in a special stabilizing solution. **Calibrated to NIST SRM 921.**

Kit 1C3690 / 1 or 1C3692 / 3	125 or 625 µL	Catalog Number 1C369b or 1C369n

Cortisol Antibody
A mouse monoclonal antibody specific for cortisol.

Kit 1C3690 / 1 or 1C3692 / 3	3 or 13mL	Catalog Number 1C369c or 1C369o

Cortisol Conjugate
A cortisol-peroxidase conjugate in a special stabilizing solution.

Kit 1C3690 / 1 or 1C3692 / 3	3 or 13mL	Catalog Number 1C369d or 1C369p

Assay Buffer (or Concentrate)
One plate kit uses a ready-to-use Assay Buffer. Five plate kit uses a 5X concentrate that should be diluted with deionized or distilled water.

Kit 1C3690 / 1	28 mL	Catalog Number 1C369e
Kit 1C3692 / 3	55 mL (Conc)	Catalog Number 1C369q

Dissociation Reagent

Kit 1C3690 / 1 or 1C3692 / 3	1 or 5 mL	Catalog Number 1C369f or 1C369r

Allow to warm completely to **Room Temperature** prior to use.

Dissociation Reagent is to be used only with Serum and Plasma samples.

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.

Kit 1C3690 / 1 or 1C3692 / 3	30 or 125mL	Catalog Number 1C062g or 1C369s

TMB Substrate

Kit 1C3690 / 1 or 1C3692 / 3	1 1 or 55 mL	Catalog Number 1C787f or 1C369t

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.

Kit 1C3690 / 1 or 1C3692 / 3	5 or 25 mL	Catalog Number1C787g or 1C369u

Plate Sealer

Kit K003-H1/H1W or 1C3692 / 3	1 or 5 Each	Catalog Number 1C362j

Storage Instructions

All components of this kit should be stored at 4°C until the expiration date of the kit.

Other Materials Required

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25µL, 50 µL and 100 µL.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free** Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Sample Types

This assay has been validated for saliva, urine, serum and EDTA and heparin plasma samples and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit.

Cortisol is identical across all species and we expect this kit may measure cortisol from sources other than human. The end user should evaluate recoveries of cortisol in other samples being tested.

Sample Preparation

Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Addition of this reagent will yield the total cortisol concentration in serum or plasma. **Dissociation Reagent is to be used only with Serum and Plasma samples.** Free cortisol can be measured in saliva and urine samples as directed below.

Dried Fecal Samples

The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Serum and Plasma Samples

Serum and plasma samples should be diluted with an equal volume of the supplied Dissociation Reagent. The diluted samples should then be further diluted ≥ 1:50 with the supplied Assay Buffer prior running in the assay. Final serum and plasma dilutions will be ≥ 1:100.
Allow the Dissociation Reagent to warm completely to **Room Temperature** before use.

NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Saliva Samples

Saliva samples should be diluted ≥ 1:4 or greater with the supplied Assay Buffer prior running in the assay.

Urine Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay. Urinary cortisol normally ranges from 0.7-119µg/gram[8] of creatinine or approximately 100,000 to 1,000,000 pg/mL[9] in 24 hour urine samples. Samples may need to be diluted substantially to read within the standard curve range.

Tissue Culture Media

For measuring cortisol in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all Samples within 2 Hours of preparation.

8. "82920 Clinical: Cortisol, Free, Random, Urine." Mayo Medical Laboratories: Reference Laboratory services for hospitals worldwide. Web. 04 Aug. 2009. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/82920>.
9. Tietz, NW, In "Textbook of Clinical Chemistry", WB Saunders, 1986.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine cortisol concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer (for 1C3692/3)

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable for 3 months at 4°C.

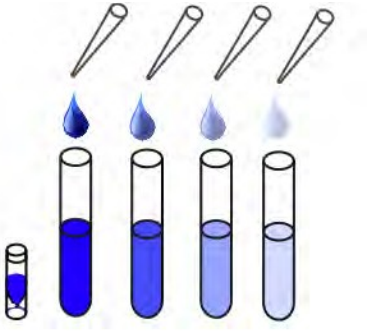
Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months at room temperature.

Standard Preparation

Label six test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #6. **The cortisol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the cortisol stock solution to tube #1 and vortex completely. Take 250 µL of the cortisol solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of cortisol in tubes 1 through 6 will be 3,200, 1,600, 800, 400, 200, and 100 pg/mL.

Use all Standards within 2 hour of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer Volume (µL)	**450**	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**50**	250	250	250	250	250
Final Conc (pg/mL)	3,200	1,600	800	400	200	100

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. If you are using the 1 by 8 well strip plate version of the kit, 1C369/0 or /2, determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 50 µL of samples or standards into wells in the plate.

3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.

4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).

5. Add 25 µL of the Cortisol Conjugate to each well using a repeater pipet.

6. Add 25 µL of the Cortisol Antibody to each well, **except the NSB wells** , using a repeater pipet.

7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 1 hour.

8. Aspirate the plate and wash each well 4 times with 300µL wash buffer. Tap the plate dry on clean absorbent towels.

9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

10. Incubate the plate at room temperature for 30 minutes without shaking.

11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

13. Use the plate reader's built-in 4PLC software capabilities to calculate cortisol concentration for each sample.

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 for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

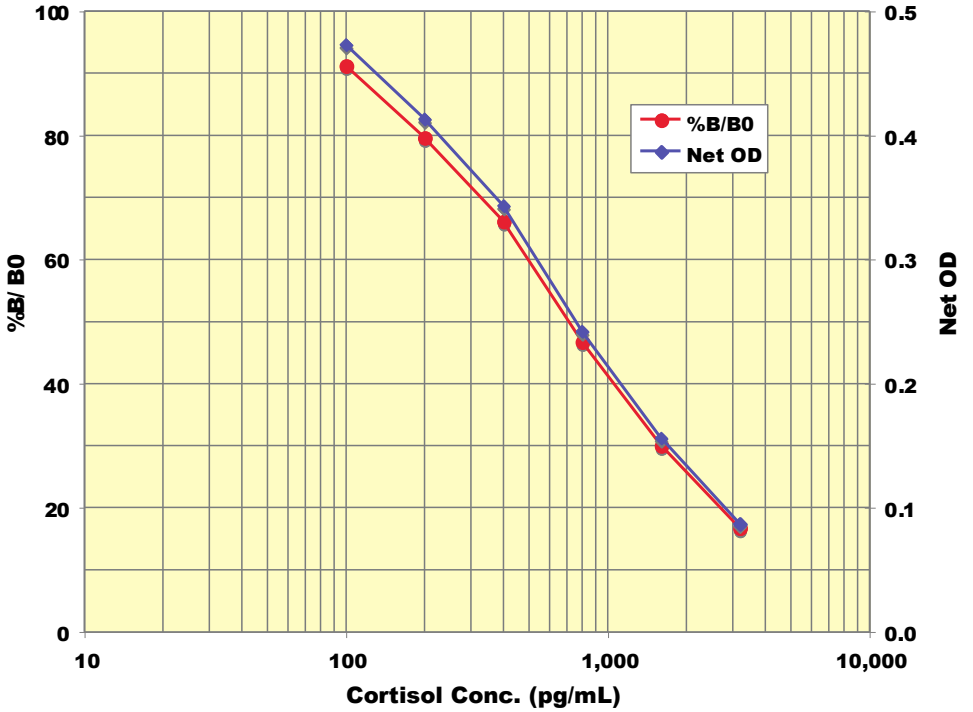
Typical Data

Sample	Mean OD	Net OD	% B/B0	Cortisol Conc. (pg/mL)
NSB	0.044	0	-	-
Standard 1	0.131	0.087	16.7	3,200
Standard 2	0.200	0.156	30.1	1,600
Standard 3	0.286	0.242	46.7	800
Standard 4	0.387	0.343	66.2	400
Standard 5	0.457	0.413	79.7	200
Standard 6	0.517	0.473	91.3	100
B0	0.562	0.518	100.0	0
Sample 1	0.137	0.093	18.0	2974.9
Sample 2	0.478	0.434	83.8	163.9

Always run your own standard curve for calculation of results.
Do not use this data.

Conversion Factor: 100 pg/mL of cortisol is equivalent to 275.9 pM.

Typical Standard Curves



Always run your own standard curves for calculation of results.
Do not use this data.

Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 17.3 pg/mL.

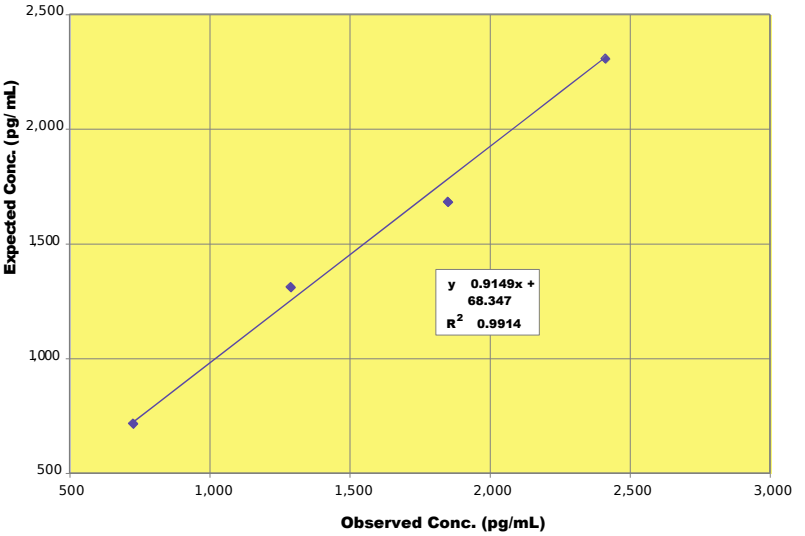
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 45.4 pg/mL

Linearity

Linearity was determined by taking two human urine samples diluted 1:140, one with a low diluted cortisol level of 163.9 pg/mL and one with a higher diluted level of 2,974.9 pg/mL and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Urine	High Urine	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
100%	0%	163.9	--	--
80%	20%	715.7	726.1	98.6
60%	40%	1,311.5	1,288.3	101.8
40%	60%	1,683.3	1,850.5	91.0
20%	80%	2,306.3	2,412.7	95.6
0%	100%	2,974.9	--	--
			Mean Recovery	**96.7%**



Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Cortisol concentrations were:

Sample	Cortisol Conc. (pg/mL)	%CV
1	1,174.3	6.0
2	475.9	5.6
3	177.4	14.7

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by four operators. The mean and precision of the calculated Cortisol concentrations were:

Sample	Cortisol Conc. (pg/mL)	%CV
1	1,188.1	7.2
2	508.7	6.3
3	199.7	10.9

Sample Values

Six random human serum and plasma samples were tested in the assay. Neat sample values ranged from 8.5 to 23.8 µg/dL with an average of 12.2 µg/dL. The normal reference range for serum cortisol is 3-23 µg/dL [9]. Four random human urine samples were tested in the assay. Neat sample values ranged from 98.1 to 304.9 µg/g creatinine with an average of 159.8 µg/g creatinine. Creatinine levels were determined using the FluoProbes Creatinine kits.

Dried fecal samples were processed as described on page 7 and run in the assay. Samples kindly donated by Dr. J. Williams at the Indianapolis Zoo, which included Amur Tiger, Giraffe, Kudu, Lion, Reeves Muntjac, White Handed Gibbon, White Rhino, and Zebra, were tested and cortisol values obtained ranged from 2.48 to 27.22 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered cortisol is excreted in differing amounts in urine and feces[10] across species, with fecal excretion ranging from 7% of administered cortisol in the pig to 82% in the cat[11,12,13]. Palme has also shown that the peak of fecal cortisol concentrations occur at 12 hours for sheep, but takes 48 hours to peak in pigs. It is therefore necessary to evaluate the timing and relative fecal or urine excretion of glucocorticoids for each species.

10. Möstl, E., et al, Vet. Res. Commun. "Measurement of Cortisol Metabolites in Faeces or Ruminants." 2002, 26:127-139.
11. Palme, R., et al, Animal Reprod. Sci., "Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock." 1996, 43:43-63.
12. Teskey-Gerstl, A., et al, J. Comp. Physiol. B, "Excretion of corticosteroids in urine and faeces of hares (Lepus europaeus)." 2000, 170: 163-168.
13. Schatz, S. and Palme, R., Vet. Res. Commun., Measurement of Faecal Cortisol Metabolites in Cats and Dogs: A Non-Invasive Method for Evaluating Adrenocortical Function.", 2001, 25:271-287.

Cross Reactivity

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

Steroid	Cross Reactivity (%)
Cortisol	100%
Dexamethasone	18.8%
Prednisolone (1-Dehydrocortisol)	7.8%
Corticosterone	1.2%
Cortisone	1.2%
Progesterone	<0.1%

Limited Warranty

FluoProbes warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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