ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Corticosterone
Enzyme Immunoassay Kit

1 or 5 Strip Plates Catalog Number K014-H1/H5

Species Independent

Multi-Format Kit

Sample Types Validated:

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

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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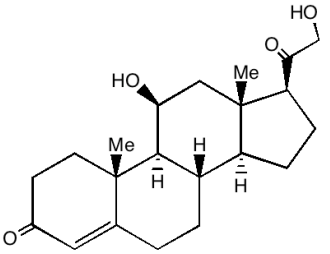
BACKGROUND

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. Corticosterone is a major indicator of stress and is the major stress steroid produced in non-human mammals. Studies involving corticosterone and levels of stress include impairment of long term memory retrieval[1], chronic corticosterone elevation due to dietary restrictions[2] and in response to burn injuries[3]. In addition to stress levels, corticosterone is believed to play a decisive role in sleep-wake patterns[4,5].



1. Hupé, JM, et al "Cortical feedback improves discrimination between figure and background by V1, V2 and V3 neurons." Nature, 1998; 394: 784-787.
2. Kitaysky AS, Kitaiskaia EV, Wingfield JC, Piatt JF. "Dietary restrictions causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks." J. Comp. Physiol, 2001; 171: 701-709.
3. Thellin O, Noel G, Khuana S, Ogle CK and Horseman ND "Stress hormone secretion and gut signal transducer (STAT) proteins after burn injury in rats." Shock, 2001; 16(5): 393-397.
4. Krame, KM. and Sothern RB. "Circadian characteristics of corticosterone secretion in red-backed voles (Clethrionomys gapperi)." Chronobiol. Int., 2001; 18(6): 933-945.
5. Vazquez-Palacios G, et al, "Further definition of the effect of corticosterone on the sleep-wake pattern in the male rat." Pharmacol. Biochem Behavior, 2001: 70(2-3): 305-310.

ASSAY PRINCIPLE

The DetectX® Corticosterone Immunoassay kit is designed to quantitatively measure Corticosterone present in serum, plasma, saliva, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total corticosterone in serum and plasma and in extracted fecal samples.

The kit offers two standard curve ranges. Depending on the anticipated sensitivity needed for your samples, you can use 50 µL of standads and samples with an assay range of 10,000 pg/mL to 39.063 pg/mL or use 100 µL of standards and samples with an assay range of 5,000 to 19.53 pg/mL. A corticosterone stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Please choose the standard range that fits your sample concentrations most appropriately.

Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A corticosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to corticosterone to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound corticosterone-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 450nm wavelength. The concentration of the corticosterone 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	Catalog No.
Adrenocorticotropic Hormone (ACTH) ELISA Kits	K072-H1/H5
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Cortisol ELISA Kits	K003-H1/H5
Cortisone Chemiluminescent ELISA Kits	K017-C1/C5
Cortisone ELISA Kits	K017-H1/H5
Hemoglobin High Sensitivity Detection Kits	K013-HX1/HX5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

Kit K014-H1 **or** -H5	1 **or** 5 Each	Catalog Number X061-1EA, 1 x 8 Strip Well

Corticosterone Standard
Corticosterone at 100,000 pg/mL in a special stabilizing solution.

Kit K014-H1 **or** -H5	125 µL **or** 625 µL	Catalog Number C151-125UL **or** -625UL

DetectX® Corticosterone Antibody
A sheep polyclonal antibody specific for corticosterone.

Kit K014-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C044-3ML **or** -13ML

DetectX® Corticosterone Conjugate
A corticosterone-peroxidase conjugate in a special stabilizing solution.

Kit K014-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C045-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that must be diluted with deionized or distilled water.

Kit K014-H1 **or** -H5	28 mL **or** 55 mL	Catalog Number X065-28ML **or** -55ML

Dissociation Reagent

Kit K014-H1 **or** -H5	1 mL **or** 5 mL	Catalog Number X058-1ML **or** -5ML

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 K014-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K014-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K014-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K014-H1 **or** -H5	1 **or** 5 Each	Catalog Number X002-1EA

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.

Organic solvent for fecal extraction and equipment for drying down (such as SpeedVac centrifugal concentrators).

Repeater pipet with disposable tips capable of dispensing 25, 50, 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 serum, EDTA and heparin plasma, saliva and urine 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. Hemoglobin levels can be determined using our Hemoglobin High Sensitivity Detection Kits, K013-HX1 and K013-HX5. Corticosterone can be assayed in other sample types by using one of the extraction protocols available on our website at: www.ArborAssays.com/resources/#protocols.

Corticosterone is identical across all species and we expect this kit may measure corticosterone from sources other than human. The end user should evaluate recoveries of corticosterone 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 corticosterone concentration in serum or plasma. **Dissociation Reagent is to be used only with Serum and Plasma samples.**

Serum and Plasma Samples

Allow the Dissociation Reagent to warm completely to **Room Temperature** before use. We suggest pipetting 5 µL of Dissociation Reagent into 1 mL Eppendorf tubes. Add 5 µL of serum or plasma to the Dissociation Reagent in the tube, vortex gently and incubate at room temperature for 5 minutes or longer. Dilute with 490 µL of 1X Assay Buffer. This 1:100 dilution can be diluted further with 1X Assay Buffer. Final serum and plasma dilutions should be ≥ 1:100.

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

Saliva Samples

Saliva samples should be diluted ≥ 1:2 with 1X Assay Buffer prior to running in the assay. See our Saliva Sample Handling instructions at: www.ArborAssays.com/assets/saliva-sample-protocol.pdf.

Urine Samples

Urine samples should be diluted ≥ 1:20 with 1X Assay Buffer prior to running in the assay. Please see our Urinary Creatinine Detection kits, K002-H1 and K002-H5, for assays to measure urine creatinine which can be used to allow normalization of corticosterone in a random urine specimen.

Dried Fecal Samples

We have a detailed Extraction Protocol available on our website at: www.ArborAssays.com/resources/#protocols. The ethanol concentration in the final 1X Assay Buffer dilution added to the well should be < 5%.

Tissue Culture Media

For measuring corticosterone 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, or stored at ≤ -20°C until assaying.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

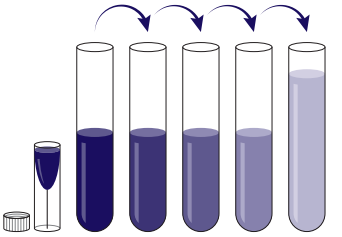
Dilute Assay Buffer Concentrate 5-fold by adding one part of the concentrate to four parts of deionized water. The 1X Assay Buffer is stable for 3 months at 4°C.

Wash Buffer

Dilute Wash Buffer Concentrate 20-fold by adding one part of the concentrate to nineteen parts of deionized water. The 1X Wash Buffer is stable for 3 months at room temperature.

Standard Preparation - 50 µL Assay Format

Label test tubes as #1 through #9. Pipet 450 µL of 1X Assay Buffer into tube #1 and 250 µL into tubes #2 to #9. **The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 250 µL of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of corticosterone in tubes 1 through 9 will be 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125 and 39.063 pg/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
1X Assay Buffer (µL)	**450**	250	250	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Vol of Addition (µL)	**50**	250	250	250	250	250	250	250	250
Final Conc (pg/mL)	10,000	5,000	2,500	1,250	625	312.5	156.25	78.125	39.063

Standard Preparation - 100 µL Assay Format

Label test tubes as #1 through #9. Pipet 570 µL of 1X Assay Buffer into tube #1 and 300 µL into tubes #2 to #9. **The corticosterone stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 30 µL of the corticosterone stock solution to tube #1 and vortex completely. Take 300 µL of the corticosterone solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of corticosterone in tubes 1 through 9 will be 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125, 39.063 and 19.531 pg/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
1X Assay Buffer (µL)	**570**	300	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Vol of Addition (µL)	**30**	300	300	300	300	300	300	300	300
Final Conc (pg/mL)	5,000	2,500	1,250	625	312.5	156.25	78.125	39.063	19.531

Use all Standards within 2 hours of preparation.

ASSAY PROTOCOL - 50 µL AND 100 µL ASSAY FORMAT

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine corticosterone concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL (100 µL for alternative format) of samples or standards into wells in the plate.
4. Pipet 75 µL (125 µL for alternative format) of 1X Assay Buffer into the non-specific binding (NSB) wells.
5. Pipet 50 µL (100 µL for alternative format) of 1X Assay Buffer into the maximum binding (B0 or Zero standard) wells.
6. Add 25 µL of the DetectX® Corticosterone Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Corticosterone Antibody to each well, except the NSB wells, using a repeater pipet.
8. 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. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 45% lower.
9. Aspirate the plate and wash each well 4 times with 300 µL 1X Wash Buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate corticosterone concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

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.

Or use these online tools from MyAssays to calculate the data:
50 µL
https://myassays.com/arbor-assays-corticosterone-enzyme-immunoassay-kit-improved-sensitivity.assay

100 µL
https://myassays.com/arbor-assays-corticosterone-enzyme-immunoassay-kit-high-sensitivity.assay

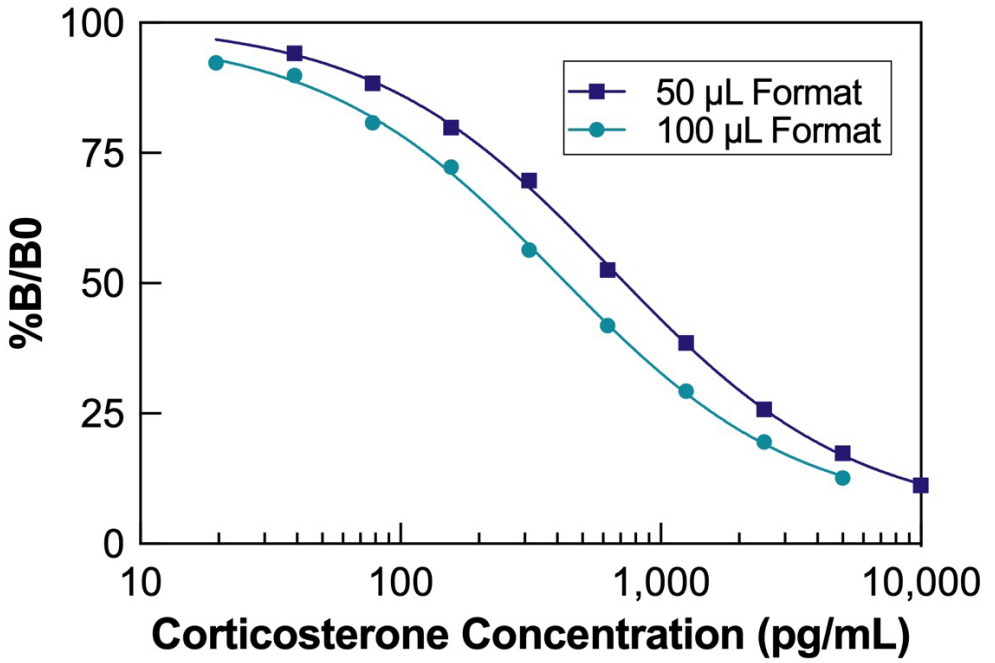
TYPICAL DATA

	50 µL Assay				100 µL Assay			
Sample	**Mean OD**	**Net OD**	**% B/B0**	**Corticosterone. Conc. (pg/mL)**	**Mean OD**	**Net OD**	**% B/B0**	**Corticosterone. Conc. (pg/mL)**
NSB	0.087	0	-	-	0.094	0	-	-
Standard 1	0.222	0.135	11.2	10,000	0.210	0.116	12.6	5,000
Standard 2	0.297	0.210	17.4	5,000	0.274	0.180	19.5	2,500
Standard 3	0.398	0.311	25.8	2,500	0.364	0.270	29.3	1,250
Standard 4	0.550	0.463	38.5	1,250	0.480	0.386	41.9	625
Standard 5	0.719	0.632	52.5	625	0.614	0.520	56.4	312.5
Standard 6	0.926	0.839	69.7	312.5	0.761	0.667	72.3	156.3
Standard 7	1.049	0.962	79.9	156.3	0.839	0.745	80.8	78.13
Standard 8	1.151	1.064	88.4	78.13	0.923	0.829	89.9	39.06
Standard 9	1.220	1.133	94.1	39.06	0.945	0.851	92.3	19.53
B0	1.291	1.204	100.0	0	1.016	0.922	100.0	0
Sample 1	0.437	0.350	29.1	2051.74	0.441	0.347	37.6	776.2
Sample 2	0.863	0.776	64.4	379.29	0.595	0.501	54.3	357.0

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

Conversion Factor: 100 pg/mL of corticosterone is equivalent to 288.6 pM.

Typical Normal Range Standard Curves



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

VALIDATION DATA

Sensitivity and Limit of Detection

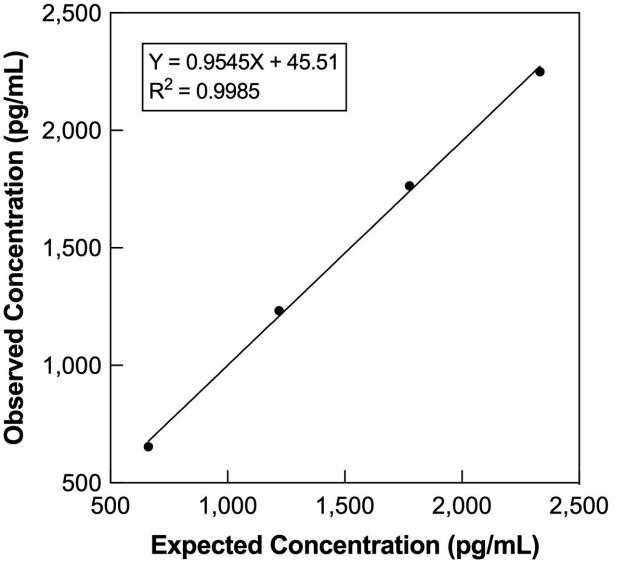
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #9. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 20.9 pg/mL for 50 µL and 14.35 pg/mL for 100 µL sample size.**

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 17.5 pg/mL for 50 µL and 7.7 pg/mL for 100 µL sample size.**

Linearity

Linearity was determined in 50 µL assay format by taking two serum samples treated with Dissociation Reagent and diluted 1:50 with 1X Assay Buffer, one with a low diluted corticosterone level of 104.6 pg/mL and one with a higher diluted level of 2,890.5 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 Serum	High Serum	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%**



Intra Assay Precision - 50 µL Assay Format

Four human samples were diluted with 1X Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Corticosterone concentrations were:

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

Inter Assay Precision - 50 µL Assay Format

Three human samples were diluted with 1X Assay Buffer and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated Corticosterone concentrations were:

Sample	Corticosterone Conc. (pg/mL)	%CV
1	2,618.3	7.5
2	630.1	6.4
3	267.9	9.9

SAMPLE VALUES

Six random mammalian serum and plasma samples were tested in the assay. Neat sample values ranged from 0.87 to 38.5 µg/dL with an average for the human samples of 1.56 µg/dL. The normal reference range for serum corticosterone is 0.13-2.3 µg/dL[6].

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 corticosterone values obtained ranged from 7.85 to 81.6 pg/mg dried fecal material.

Palme and Möestl and colleagues have shown that radiolabeled administered glucocorticoids are excreted in differing amounts in urine and feces[7] across species, with fecal excretion ranging from 7% of administered cortisol in the pig to 82% in the cat[8-10]. Palme has also shown that the peak of fecal glucocorticoid 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.

6. Tietz, NW, In "Textbook of Clinical Chemistry", WB Saunders, 1986.
7. Möstl, E., et al, Vet. Res. Commun. "Measurement of Cortisol Metabolites in Faeces or Ruminants." 2002, 26:127-139.
8. 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.
9. Teskey-Gerstl, A., et al, J. Comp. Physiol. B, "Excretion of corticosteroids in urine and faeces of hares (Lepus europaeus)." 2000, 170: 163-168.
10. 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 (%)	Steroid	Cross Reactivity (%)
Corticosterone	100%	Testostrone	0.03%
1-dehydrocorticosterone	18.9%	Corticosterone-21-hemisuccinate	< 0.1%
Desoxycorticosterone	12.3%	Cortisone	< 0.08%
1α-hydroxycorticosterone	3.3%	Estradiol	< 0.08%
11-dehydrocorticosterone	2.4%	17-hydroxyprogesterone	< 0.01%
Tetrahydrocorticosterone	0.76%	Allopregnanolone	< 0.01%
Aldosterone	0.62%	Dehydroepiandrosterone sulfate	< 0.01%
Cortisol	0.38%	Estrone-3-glucuronide	< 0.01%
Progesterone	0.24%	Estrone-3-sulfate	< 0.01%
Dexamethasone	0.12%		

LIMITED WARRANTY

Arbor Assays 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.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.