ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Adrenocorticotropic Hormone (ACTH)
Enzyme Immunoassay Kit

1 Plate Kit	Catalog Number K072-H1
5 Plate Kit	Catalog Number K072-H5

Sample Types Validated:

Plasma

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

www.ArborAssays.com

WEB K072-H 240311

TABLE OF CONTENTS

BACKGROUND

Adrenocorticotropic hormone (ACTH, also adrenocorticotropin, corticotropin) is a 39 amino acid polypeptide hormone (MW=4,500) secreted by the pituitary to regulate the production of steroid hormones by the zona fasciculata region of the adrenal cortex. ACTH is an important component of the hypothalamic-pituitary-adrenal axis (HPA) produced in response to biological stress. Its principal effects are increased production and release of glucocorticoids (GCs). Stress-induced secretion of the peptide hormone Corticotropin Releasing Hormone (CRH) stimulates pituitary ACTH secretion. Circulating ACTH binds to melanocortin receptors on the surface of adrenal zona cells inducing the synthesis and release of all adrenal steroids, aldosterone, GCs and adrenal androgens. ACTH is the principal modulator of cortisol and corticosterone, considered the most important glucocorticoids in higher organisms. As circulating cortisol or corticosterone levels increase, further release of ACTH is inhibited by a negative feedback control mechanism at the pituitary level, leading to a reduction in cortisol and corticosterone levels.[2,3,4,5]

In addition to the stress response, ACTH synthesis is related to the circadian rhythm in many organisms. In healthy individuals, circulating ACTH levels peak in the early morning and taper off to the lowest levels just before sleep, though stress may override the diurnal variation. Plasma ACTH levels are useful in the diagnosis of pituitary Cushing's disease[6], Addison's disease, pituitary tumors, hypopituitarism, and ectopic ACTH syndrome.

Please refer to Cross Reactivity on page 14 to learn more about applicability to other species.

1. Watts, N. B. and Keffer, J. H. (1982). *Practical Endocrine Diagnosis* (3rd ed.). Philadelphia, PA: Lea and Febioer
2. Ganong, W. F., et al. (1974). ACTH and the regulation of adrenocorticol secretion. *New England Journal of Medicine, 290*(18), 1006–1011.
3. Tepperman, J. (1981). *Metabolic and Endocrine Physiology* (4th ed.). Yearbook Medical Publishers, Inc.
4. Odell, W. D., et al. (1989). The use of ACTH and cortisol assays in the diagnosis of endocrine disorders. *Nichols Institute Publication*, 249–259.
5. Nichols, A. L. and Nelson, J. C. (1977). *Radioimmunoassay Manual* (4th ed.). Nichols Institute.
6. Raff, Hershel and Carrol, Ty (2015). Cushing's Syndrome: From physiological principles to diagnosis & clinical care. *The Journal of Physiology, 1:593*(3), 493–506.

ASSAY PRINCIPLE

The DetectX® Adrenocorticotropic hormone (ACTH) Kit is designed to quantitatively measure ACTH present in plasma samples. Please read the complete kit insert before performing this assay. An ACTH standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

Standards or undiluted plasma samples are added to a clear microtiter plate coated with a monoclonal antibody to capture ACTH present in the sample. After a 60-minute incubation, the plate is washed and a peroxidase-conjugated ACTH monoclonal antibody is added. The plate is incubated for 30 minutes, followed by a wash. Substrate is added, which reacts with the bound ACTH-conjugated antibody. After 30 minutes, the substrate 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 ACTH in the sample is calculated, after making suitable correction for any dilution, using software available with most plate readers.

RELATED PRODUCTS

DetectX® Kits	Catalog No.
Aldosterone Chemiluminescent ELISA Kits	K052-C1/C5
Aldosterone ELISA Kits	K052-H1/H5
Androstenedione ELISA Kits	K070-H1/H5
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Corticosterone ELISA Kits	K014-H1/H5
Cortisone Chemiluminescent ELISA Kits	K017-C1/C5
Cortisone ELISA Kits	K017-H1/H5
Cortisol ELISA Kits	K003-H1/H5
Dehydroepiandrosterone Sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Epiandrosterone ELISA Kits	K063-H1/H5
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Testosterone ELISA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Mouse Anti-ACTH Clear Coated 96 Well Plate

Clear plastic microplate with break-apart strips coated with mouse anti-human adrenocorticotropic hormone.

K072-H or -H5	1 or 5 Each	Catalog Number C271-1EA

Human ACTH Standard

Lyophilized human ACTH at 1,000 pg stored in a ziplock pouch with desiccant.

K072-H1 or -H5	2 or 5 Each	Catalog Number C270-1EA

DetectX® ACTH Conjugate

A mouse anti-human monoclonal antibody to adrenocorticotropic hormone labeled with peroxidase.

K072-H1 or -H5	5 mL or 25 mL	Catalog Number C269-5ML or -25ML

Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.

K072-H1 or -H5	28 mL or 80 mL	Catalog Number X122-28ML or -80ML

Wash Buffer Concentrate

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

K072-H1 or -H5	30 mL or 125 mL	Catalog Number X007-30ML or -125ML

TMB Substrate

K072-H1 or -H5	11 mL or 55 mL	Catalog Number X019-11ML or -55ML

Stop Solution

A 1M hydrochloric acid solution. **CAUSTIC**.

K072-H1 or -H5	5 mL or 25 mL	Catalog Number X020-5ML or -25ML

Plate Sealer

K072-H1 or -H5	2 or 10 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.

Polypropylene test tubes or vials for preparing standards and sample dilutions. **Do not use glass.**

A microplate washer.

Repeater pipet with disposable tips capable of dispensing 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.

Protease Inhibitor as indicated in Sample Preparation on page 7 for long-term storage of plasma samples.

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 standards and antibody-coated plate need to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the standard and 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 human plasma samples only. Samples containing visible particulate should be centrifuged prior to using. The end user should test this kit for application in their samples.

Please refer to Cross Reactivity on page 14 to learn more about applicability to other species.

SAMPLE PREPARATION

Plasma Samples

Plasma samples are run neat in the assay. If extremely elevated levels are expected, as seen in certain disease states as shown on page 14, dilution in Assay Buffer may be necessary.

Collection

For the 12 hours before specimen collection, do not take multivitamins or dietary supplements containing biotin (vitamin B7), which is commonly found in hair, skin, and nail supplements and multivitamins.

Morning (6:00–10:30) specimen collection is desirable. ACTH is at its lowest concentration at 23:00 hours.

Collect with a pre-chilled blood collection tube.

Centrifuge at refrigerated temperature within 2 hours and immediately separate plasma. Keep plasma chilled or on ice until used or prepared for longer-term storage.

Storage of Samples

Samples can be stored at room temperature for up to 2 hours, or up to 3 hours if stored on ice. Store plasma ≤ -20°C for up to 28 days after addition of Protease Inhibitors.

The following Protease Inhibitors MUST be added to all plasma samples prior to long-term storage:

- Phenylmethanesulfonyl fluoride (PMSF), such as Sigma 78830 at 100 mM in ethanol, diluted to 1 mM in samples.
- A universal protease inhibitor cocktail (PIC), such as Sigma P1860 or Roche 05892970001, diluted 1:200 in samples, or according to manufacturer's instructions.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Prepare all ACTH dilutions in polypropylene tubes or vials. **Do not use glass.**

Assay Buffer

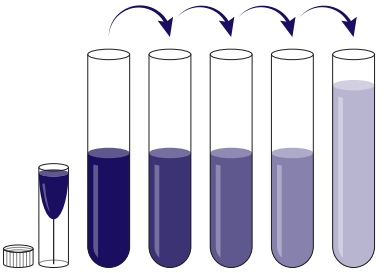
Dilute Assay Buffer Concentrate 1:5 by adding 1 part of the concentrate to 4 parts of deionized water. Once diluted, this is stable for 3 months at 4°C.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to 19 parts of deionized water. Once diluted, this is stable at room temperature for 3 months.

Standard Preparation

Allow the ziplock bag to warm to room temperature prior to opening. Remove 1 vial of standard and add 100 µL of distilled or deionized water to the vial to generate the 10,000 pg/mL Standard Stock. Allow to sit at room temperature for 5 minutes. Vortex the vial. **Standard Stock is stable at 4°C for up to one week.** Label test tubes as #1 through #7. Pipet 270 µL of Assay Buffer into tube #1. Pipet 150 µL of Assay Buffer into tubes #2 to #7. Carefully add 30 µL of Standard Stock to tube #1 and vortex completely. Take 150 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of ACTH in tubes #1 through #7 will be 1,000, 500, 250, 125, 62.5, 31.25, and 15.625 pg/mL.



	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	**diH_2O**	270	150	150	150	150	150	150
Addition	Vial	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	**100**	30	150	150	150	150	150	150
Final Conc (pg/mL)	10,000	1,000	500	250	125	62.5	31.25	15.625

Use all Standards within 2 hour of preparation. Discard diluted standards. Reconstituted Standard Stock can be stored at 4°C for up to one week.

Prepare all reagents, standards and samples prior to starting the assay. ACTH binding to the plate antibody starts as soon as samples or standards are added to the well.

ASSAY PROTOCOL

Ensure that all chilled samples have been diluted if needed and have reached room temperature just prior to running them in the kit.

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine ACTH 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 foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
3. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells.
4. Cover the plate with the plate sealer.
5. **Assay binding reaction starts as soon as the first sample or standard is added.** Incubate at room temperature for 60 minutes with shaking. We recommend shaking at around 700–900 rpm. Non-shaking will reduce ODs by approximately 20%.
6. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer. Tap the plate dry on clean, absorbent towels.
7. Add 50 µL of the DetectX® ACTH Conjugate to each well using a repeater pipet.
8. Cover the plate with the plate sealer and shake at room temperature for **30 minutes.**
9. Aspirate the plate and wash each well 4 times with 300 µL of diluted 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 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 ACTH 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 computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations are for neat sample values, unless dilutions were prepared.

Or, use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-detectx-adrenocorticotropic-hormone-(acth).assay

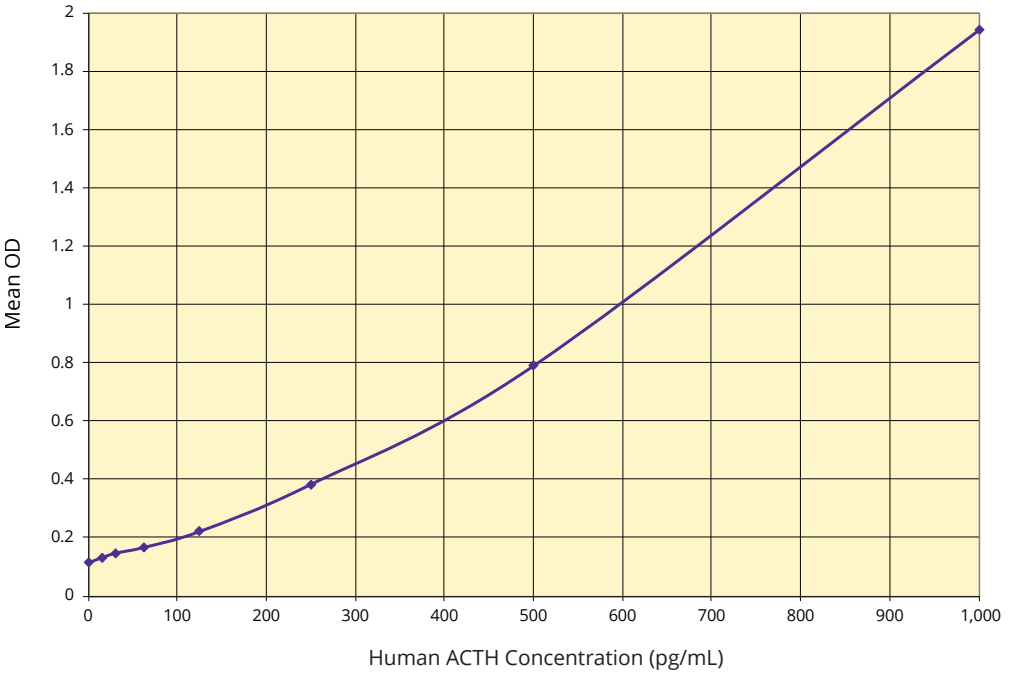
TYPICAL DATA

Sample	Mean OD	ACTH Conc. (pg/mL)
Standard 1	1.941	1,000
Standard 2	0.7895	500
Standard 3	0.383	250
Standard 4	0.2215	125
Standard 5	0.167	62.5
Standard 6	0.1475	31.25
Standard 7	0.132	15.625
Zero	0.114	0
Sample 1	0.313	204.0
Sample 2	0.551	363.1

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

Conversion of 1 pg/mL equals 0.22 pmol/L.

Typical 2 Hour Standard Curve



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

VALIDATION DATA

Sensitivity and Limit of Detection

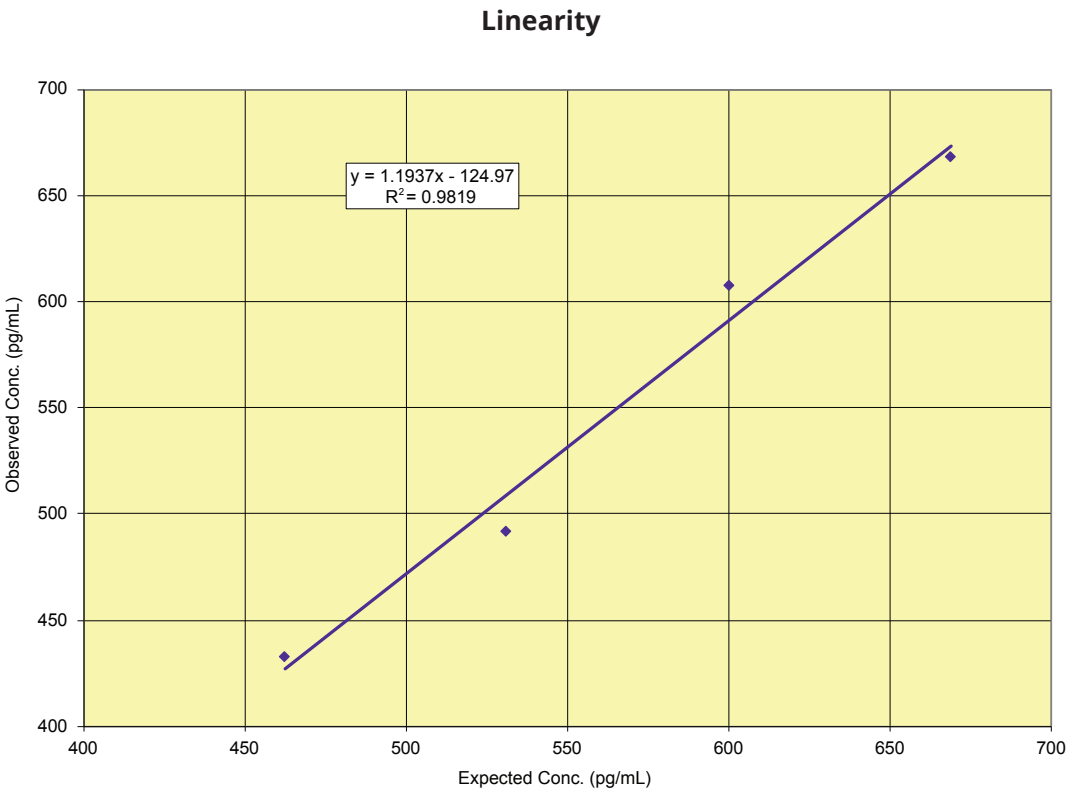
Sensitivity was calculated by comparing the OD's for 18 wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero standard along the standard curve. Sensitivity was determined as 12.71 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for 19 runs for each of the zero standard and a low concentration human sample. Limit of Detection was determined as 10.69 pg/mL.

Linearity

Linearity was determined by taking two plasma samples, one with a low ACTH level of 393.5 pg/mL and one with a higher level of 737.6 pg/mL and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Plasma	Low Plasma	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	668.8	668.0	99.9
60%	40%	600.0	607.7	101.3
40%	60%	531.1	492.1	92.6
20%	80%	462.3	432.7	93.6
			Mean Recovery	**96.9%**



Intra Assay Precision

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

Sample	ACTH Conc. (pg/mL)	%CV
1	164.0	9.6
2	299.0	7.4
3	709.2	2.2

Inter Assay Precision

Three samples were diluted with Assay Buffer and run in duplicates in sixteen assays run over multiple days by several operators. The mean and precision of the calculated ACTH concentrations were:

Sample	ACTH Conc. (pg/mL)	%CV
1	188.0	12.4
2	317.7	11.4
3	724.2	12.4

High Dose Hook

Samples were prepared with ACTH concentrations as high as 1,000,000 pg/mL and tested in the assay. Bound optical density levels indicated that high ACTH concentrations would not give artificially depressed ACTH concentrations.

SAMPLE VALUES

Reference ranges for humans (a.m. draw) are 7.2–63 pg/mL, per Mayo Clinic Laboratories.[1] No difference was seen with pediatric reference ranges. Normal basal ACTH values for children 4–16 years of age were 11–82 pg/mL. Normal values were also found in girls with precocious puberty and in children with McCune-Albright and Beckwith-Wiedemann's syndromes. In untreated children with congenital adrenal hyperplasia, values were between 233 and 305 pg/mL. In primary Addison's disease, levels were very high (654–1,413 pg/mL) before cortisone therapy and declined to nearly normal values during therapy. ACTH values in a case of pituitary Cushing's syndrome were above the normal range at 104–163 pg/mL.[2]

1. Mayo Clinic Laboratories. Test ID: ACTH. Adrenocorticotropic Hormone, Plasma. (https://www.mayocliniclabs.com/test-catalog/clinical+and+interpretive/8411).
2. Petersen, K. E. (1981). ACTH in normal children and children with pituitary and adrenal diseases. I: Measurement in plasma by radioimmunoassay—basal values. *Acta Paediatrica, 70*(3), 341–345.

CROSS REACTIVITY

ACTH is the 39 amino acid post-translational fragment of the POMC protein. The sequence of ACTH is highly conserved in mammals, such as macaque, sheep, pigs, horse, etc. from 97.4–100%. Sequence similarity between human and rodents (mice and rats) is lower at 94.8%, and this reduces the cross reactivity of these rodent ACTH molecules. However, researchers should perform and rely on cross reactivity with validation experiments to determine if the kit has the ability to read ACTH in species other than humans.

The following cross reactants were tested in the assay and calculated on the standard curve.

Steroid	Cross Reactivity (%)
human ACTH	100.00%
mouse ACTH	22.10%
POMC	1.57%
alpha-MSH	<0.1%
ACTH 18–39	<0.1%
ACTH 1–24	<0.1%
ACTH 1–10*	<0.1%
ACTH 4–10*	<0.1%
ACTH 1–13*	<0.1%
ACTH 1–18*	<0.1%
β-LPH*	<0.1%
*Estimated based upon RIA assay data	

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

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

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 EIA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12