ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

HIGH SENSITIVITY RBP
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number KU04-H1
5 Plate Kit Catalog Number KU04-H5

Species Independent

Formerly Named Urinary RBP EIA Kit

Assay Buffer Change: See pages 5 & 8

Sample Types Validated:

Dried Blood Spot and Urine

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

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TABLE OF CONTENTS

BACKGROUND

Retinol binding protein (RBP) is from a family of structurally related proteins that bind small hydrophobic molecules such as bile pigments, steroids, odorants, etc[1]. RBP is a 21 kDa highly conserved, single-chain glycoprotein, consisting of 182 amino acids with 3 disulfide bonds, that has a hydrophobic pocket which binds retinol (vitamin A).

RBP binds retinol in a 1:1 stoichiometry, which serves to not only solubilize retinol but also protect it from oxidation. When in serum, the majority of RBP bound with retinol is reversibly complexed with transthyretin (prealbumin)[2,3]. This complex then transports retinol to specific receptors of various tissues in the body. Vitamin A status is reflected by serum concentration as it is hemostatically controlled and does not fall until stores are dramatically reduced[4,5].

RBP has also been shown to be a useful marker for renal function[6] as it is totally filtered by the glomeruli and reabsorbed by proximal tubules[7]. This has made urinary RBP (uRBP) a tool to study renal function in heart[8] or kidney[9] transplant recipients, type 1 and 2 diabetics[10], and in people exposed to uranium from mining operations[11]. Measurement of uRBP levels has also been useful in detection and characterization of diseases including hypertension[12] and certain cancers[13,14].

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8. Câmara NO., Matos AC., Rodrigues DA., et al., “Early Detection of Heart Transplant patients with Increased Risk of Cyclosporin Nephrotoxicity.”, The Lancet, 2001, 357: 856-857.
9. Hosaka B., Park SI., Felipe CR., Garcia RG., Machado PG., Pereira AB., Tedesco-Silva H., and Medina Pestana JO., “Predictive Value of Urinary Retinol Binding Protein for Graft Dysfunction after Kidney Transplantation.” Transplant Proc., 2003, 35:1341-1343.
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14. Gavrilov V., Yermiahu T., Gorodischer R., “Renal pathology and retinol status in multiple myeloma patients.” Kidney Int., 2006, 69(1):173-7.

ASSAY PRINCIPLE

The DetectX® High Sensitivity Retinol Binding Protein (RBP) kit is designed to quantitatively measure RBP present in dried blood spot (DBS) and urine samples. Please read the complete kit insert before performing this assay. A RBP 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 rabbit antibodies. A RBP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of the RBP polyclonal antibody to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound RBP-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 RBP in the sample is calculated, after making a suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
BCA Protein Dual Range Colorimetric Detection Kit	K041-H1
Human Cystatin C EIA Kit	K012-H1
Serum Creatinine Detection Kit	KB02-H1
Thiol Detection Kit	K005-F1
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates
A clear plastic microplate(s) with break-apart strips coated with goat anti-rabbit IgG.

Kit KU04-H1 **or** -H5 1 **or** 5 Each — Catalog Number X016-1EA

RBP Standard
A stock solution of native human RBP at 20 µg/mL.

Kit KU04-H1 **or** -H5 60 µL **or** 240 µL — Catalog Number C014-60UL **or** -240UL

DetectX® RBP Antibody
A polyclonal antibody specific for RBP.

Kit KU04-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C011-3ML **or** -13ML

DetectX® RBP Conjugate
A RBP-peroxidase conjugate.

Kit KU04-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C015-3ML **or** -13ML

Assay Buffer Concentrate *now supplied as a concentrate*
A 5X concentrate that must be diluted with deionized or distilled water.

Kit KU04-H1 **or** -H5 28 mL **or** 55 mL — Catalog Number X053-28ML **or** -55ML

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

Kit KU04-H1 **or** -H5 30 mL **or** 125 mL — Catalog Number X007-30ML **or** -125ML

TMB Substrate
Kit KU04-H1 **or** -H5 11 mL **or** 55 mL — Catalog Number X019-11ML **or** -55ML

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

Kit KU04-H1 **or** -H5 5 mL **or** 25 mL — Catalog Number X020-5ML **or** -25ML

Plate Sealer
Kit KU04-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.

A microplate shaker and a microplate washer.

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

The RBP Standard is purified from a human source and as such, should be treated as potentially hazardous. Proper safety procedures must be followed.

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.

Laboratory temperature is important. Please make sure that the kit incubates at a temperature between 22°C and 24°C.

SAMPLE TYPES

This assay has been fully validated for human urine and dried blood spot samples. Samples containing visible particulate should be centrifuged prior to using.

RBP is a highly conserved protein and we have shown that this kit may measure RBP from sources other than human. Please see page 14 for details of other urine samples tested. The end user should evaluate recoveries of RBP in other urine samples being tested.

SAMPLE PREPARATION

Urine Samples

Samples must be diluted 1:2 by adding one part of urine to one part diluted Assay Buffer prior to running in the kit. Any samples with RBP concentrations greater than the standard curve range should be diluted further with diluted Assay Buffer to obtain readings within the standard curve. Samples that are too dilute to be measured should be concentrated prior to measuring in the assay.

Dried Blood Spots (DBS)

Dried blood spot (DBS) samples should be prepared according to the 2007 Clinical Chemistry paper by Masako Fujita, et al, vol. 53 (11), page 1972-1975. Briefly, whole blood is spotted onto Whatman 309 filter paper and thoroughly dried at room temperature. These can be stored desiccated at ≤ 4°C until use. DBS samples, 1/4" or 1/8", are punched out into clean plastic tubes with caps. For each 1/4" DBS a minimum of 240 µL of diluted Assay Buffer is added. For each 1/8" DBS a minimum of 60 µL of diluted Assay Buffer is added. This is equivalent to a 1:40 dilution of the sample. The tubes are capped and left at 4°C overnight. The following morning, the red solution can be run without centrifugation or further dilution.

For calculation purposes a 1/8 inch DBS is considered to contain 1.5 µL of whole blood sample.

The dilution of any samples that fall outside the standard range should be adjusted to allow samples to read within the standard curve.

Use all samples within 2 hours of dilution.

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 *now supplied as a concentrate*

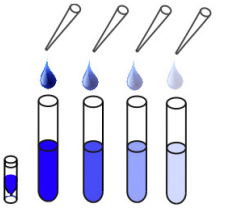
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable for 3 months at 4°C.

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.

Standard Preparation

Label tubes #1 through #5. Briefly spin vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 475 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #5. Carefully add 25 µL of the RBP stock solution to tube #1 and vortex completely. Take 100 µL of the RBP solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #5. The concentration of RBP in tubes 1 through 5 will be 1,000, 250, 62.5, 15.625, and 3.906 ng/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5
Assay Buffer Volume (µL)	**475**	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4
Volume of Addition (µL)	**25**	100	100	100	100
Final Conc (ng/mL)	1,000	250	62.5	15.625	3.906

ASSAY PROTOCOL

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

1. Use the plate layout sheet on the back page of the kit insert to aid in proper sample and standard identification. 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. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells. Pipet 50 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
3. Add 25 µL of the DetectX® RBP Conjugate to each well, using a repeater pipet.
4. Add 25 µL of the DetectX® RBP Antibody to each well, **except the NSB wells**, using a repeater pipet.
5. 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.
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 100 µL of the TMB Substrate to each well, using a repeater pipet.
8. Incubate the plate at room temperature for 30 minutes without shaking.
9. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
10. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
11. Use the plate reader's built-in 4PLC software capabilities to calculate RBP 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. Urine RBP values should be normalized to creatinine levels by running the same urines in the DetectX® Urinary Creatinine Detection Kit, K002-H1.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-retinol-binding-protein-urinary-eia-kit.assay

MyAssays

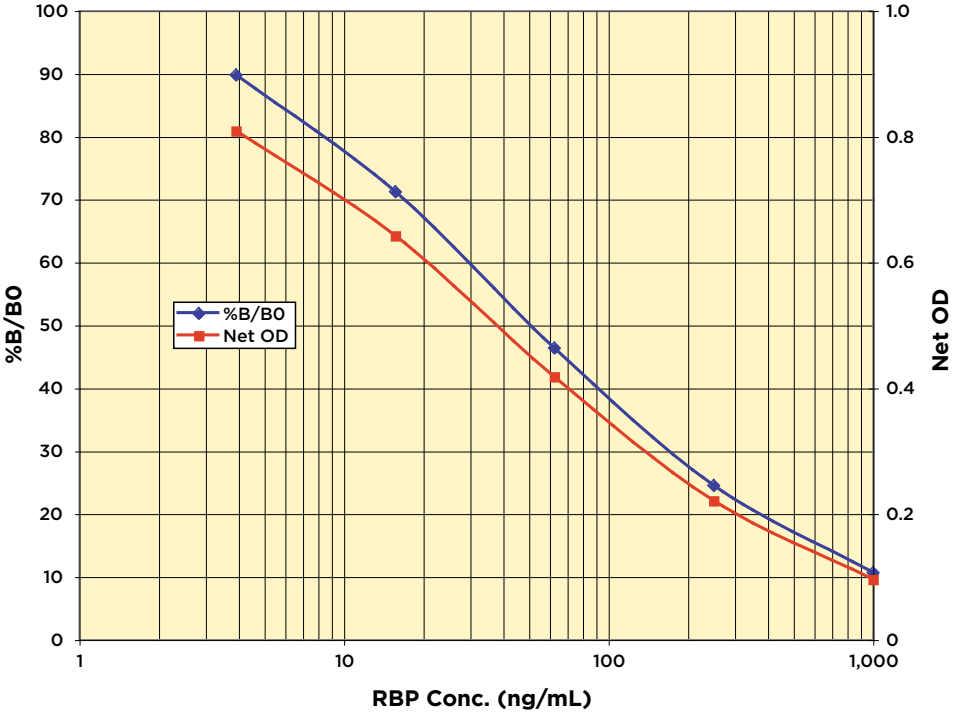
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	RBP Conc. (ng/mL)
NSB	0.098	0	-	-
Standard 1	0.194	0.096	10.7	1,000
Standard 2	0.319	0.221	24.5	250
Standard 3	0.516	0.418	46.4	62.5
Standard 4	0.740	0.642	71.3	15.625
Standard 5	0.907	0.809	89.8	3.906
B0	0.999	0.901	100	0
Sample 1	0.318	0.220	24.4	251.1
Sample 2	0.598	0.500	55.4	38.25

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

Conversion Factor: 1 ng/mL of human RBP is equivalent to 47.62 pM RBP.

Typical 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 twenty wells run for each of the B0 and standard #5. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 2.90 ng/mL.

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

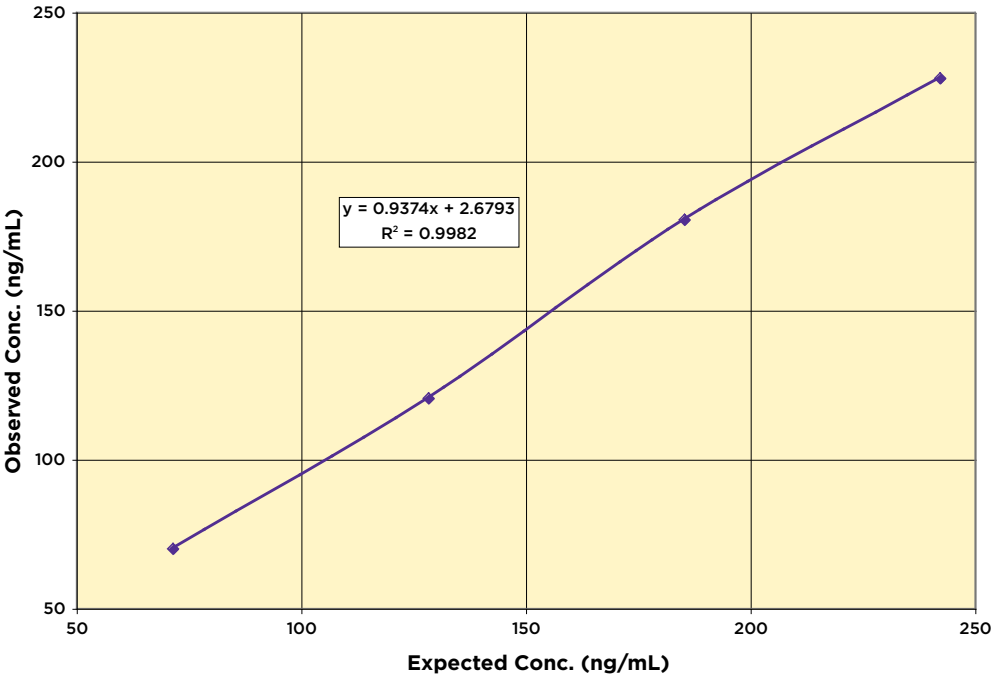
Limit of Detection was determined as 4.09 ng/mL*.

* Note: Due to the dilute nature of this sample it was run neat instead of being diluted 1:2.

Linearity

Linearity was determined by taking two human urine samples diluted 1:2, one with a low diluted RBP level of 14.5 ng/mL and one with a higher diluted level of 299.2 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	242.2	227.9	94.1
60%	40%	185.3	180.4	97.3
40%	60%	128.4	120.5	93.9
20%	80%	71.5	70.0	98.0
			Mean Recovery	**95.8%**



Intra Assay Precision

Four human urine samples were diluted 1:2 with Assay Buffer and run in replicates of 8 in an assay. The mean and precision of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	%CV
1	14.8	4.5
2	28.0	7.5
3	53.5	7.3
4	323.7	2.1

Inter Assay Precision

Four human urine samples were diluted 1:2 with Assay Buffer and run in duplicates in twenty-one assays run over multiple days by three operators. The mean and precision of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	%CV
1	14.4	9.1
2	28.0	9.2
3	50.8	8.1
4	309.2	14

SAMPLE VALUES

Fourteen random human urine samples were tested in the assay. Values ranged from 6.8 to 788.5 ng/mL with a mean of 114.7 ng/mL. These samples were also run in the DetectX® Urinary Creatinine Detection Kit, K002-H1, and the RBP levels normalized to creatinine levels. Normalized values ranged from 35.9 to 573.9 µg RBP/g creatinine.

Normal ranges for urinary RBP are < 130 µg RBP/g creatinine for individuals under 50 years of age and < 172 µg RBP/g creatinine for those equal to or older than 50[15].

Five random human whole blood DBS samples were punched out as 1/4" or 1/8" and tested in the assay. Values ranged from 7.54 to 19.7 µg/mL.

Other Species

We have tested a range of urines from other species for RBP. These include rat, dog and rhesus monkey urine. Because of the difficulty in obtaining urine from animals with known medical history, the values we obtained may not be representative of normal or diseased states. Rat urine diluted 1:2 with Assay Buffer had a neat sample value of 43.37 ng/mL and when normalized to urinary creatine gave a reading of 176.1 µg/g creatinine.

Samples of urine from healthy dog and rhesus monkey read below the 3.906 ng/mL standard. We therefore concentrated these samples by freeze drying them and reconstituting them in one-tenth their original volume with Assay Buffer. At that concentration, neat dog urine read at 29.95 ng/mL and when normalized to urinary creatine gave a reading of 32.27 µg/g creatinine. The neat monkey urine read at 10.50 ng/mL and when normalized to urinary creatine gave a reading of 395.5 µg/g creatinine.

15. 84447 Overview: Retinol-Binding Protein, Random, Urine." Mayo Medical Laboratories: Reference Laboratory services for hospitals worldwide. Web. 02 Sept. 2009. www.mayomedicallaboratories.com/test-catalog/Overview/84447

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 EIA kits for wildlife conservation research.

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FOR SERIOUS SCIENCE

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

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