



**DetectX™**

**Urinary Retinol Binding Protein  
Enzyme Immunoassay Kit**

**Catalog Number KU04-H1**

**Sample Types Validated:  
Human Urine**

**Please read this insert completely prior to using the product.**

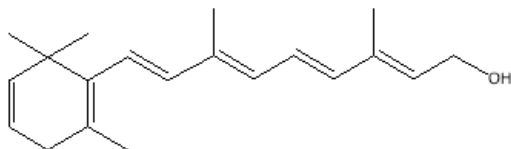
**FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROTOCOLS**

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## INTRODUCTION

Retinol binding protein (RBP) is from a family of structurally related proteins that bind small hydrophobic molecules such as bile pigments, steroids, odorants, etc<sup>1</sup>. 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). The structure of retinol is shown below.



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)<sup>2,3</sup>. 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<sup>4-5</sup>.

RBP has also been shown to be a useful marker for renal function<sup>6</sup> as it is totally filtered by the glomeruli and reabsorbed by proximal tubules<sup>7</sup>. This has made urinary RBP (uRBP) a tool to study renal function in heart<sup>8</sup> or kidney<sup>9</sup> transplant recipients, type 1 and 2 diabetics<sup>10</sup>, and in people exposed to uranium from mining operations<sup>11</sup>. Measurement of uRBP levels has also been useful in detection and characterization of diseases including hypertension<sup>12</sup> and certain cancers<sup>13-14</sup>, among other conditions<sup>15</sup>.

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**THE LUMINOS DetectX<sup>™</sup> URINARY RETINOL BINDING PROTEIN  
ENZYME IMMUNOASSAY KIT**

**Assay Principle**

The DetectX<sup>™</sup> Urinary Retinol Binding Protein (RBP) kit is designed to quantitatively measure RBP present in 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 Horseradish peroxidase-RBP 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 a 1 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound RBP-HRP 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.

## Supplied Components

<b>GxRbt Clear Microtiter Plate</b>	96 well	Catalog Number X016-1EA
A clear plastic break-apart microplate coated with goat anti-rabbit IgG.		
<b>RBP Standard</b>	60 $\mu$ L	Catalog Number C014-60UL
A stock solution of native human RBP at 20 $\mu$ g/mL.		
<b>DetectX™ RBP Antibody</b>	3 mL	Catalog Number C011-3ML
A polyclonal antibody specific for RBP.		
<b>DetectX™ RBP-HRP Conjugate</b>	3 mL	Catalog Number C015-3ML
A Horseradish peroxidase-RBP conjugate.		
<b>Assay Buffer</b>	28 mL	Catalog Number X005-28ML
<b>Wash Buffer Concentrate</b>	30 mL	Catalog Number X007-30ML
A 20X concentrate that should be diluted with deionized or distilled water.		
<b>TMB Substrate</b>	11 mL	Catalog Number X019-11ML
<b>Stop Solution</b>	11 mL	Catalog Number X020-11ML
A 1N hydrochloric acid solution. <b>Caustic.</b>		
<b>Plate Sealer</b>	1 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**

A microplate shaker and washer.

A supply of distilled or deionized water.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm, preferably with correction between 570 and 590 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 RBP Standard is purified from a human source and as such, should be treated as potentially hazardous. Proper safety procedures must be followed.

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 urine samples only. Samples containing visible particulate should be centrifuged prior to using.

RBP is a highly conserved protein and we expect this kit may measure RBP's from sources other than human. The end user should evaluate recoveries of RBP in other urine samples being tested.

For measuring retinol binding protein in serum or plasma samples Luminos also produces an immunoassay kit for serum RBP, Catalog Number K004-H1.

## **Sample Preparation**

Samples must be diluted 1:2 with the provided Assay Buffer prior to running in the kit. Any samples with RBP concentrations greater than the standard curve range should be diluted further with 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.

**Use all samples within 2 hours of dilution.**

## **Reagent Preparation**

Label five test tubes as #1 through #5. Briefly spin vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 475  $\mu\text{L}$  of Assay Buffer into tube #1 and 250  $\mu\text{L}$  into tubes #2 to #5. Carefully add 25  $\mu\text{L}$  of the RBP stock solution to tube #1 and vortex completely. Take 250  $\mu\text{L}$  of the RBP solution in tube #1 and add it to tube #2 and vortex completely. Repeat this 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.**

Prepare the Wash Buffer by diluting it 1:20 with distilled or deionized water. Once diluted it is stable for 3 months at room temperature.

## **Assay Protocol**

Allow the kit reagents to come to room temperature for 30 minutes. The recommended format is 1 hr at room temperature with shaking. The assay is sensitive to temperature. Significant changes to temperature during incubation will cause results to vary. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine RBP concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

## Assay Protocol

1. Use the plate layout sheet on page 16 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  $\mu\text{L}$  of samples or standards into wells in the plate. Pipet 75  $\mu\text{L}$  of Assay Buffer into the non-specific binding (NSB) wells. Pipet 50  $\mu\text{L}$  of Assay Buffer into wells to act as maximum binding wells (Bo).
3. Add 25  $\mu\text{L}$  of the DetectX<sup>™</sup> RBP-HRP conjugate to each well, using a repeater or multichannel pipet.
4. Add 25  $\mu\text{L}$  of the DetectX<sup>™</sup> RBP Antibody solution to each well, except the NSB wells, using a repeater or a multichannel 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 4 times with the Wash Buffer. Tap the plate dry on absorbent towels.
7. Add 100  $\mu\text{L}$  of the TMB Substrate to each well, using a repeater or a multichannel pipet.
8. Incubate the plate at room temperature for 30 minutes without shaking.
9. Add 100  $\mu\text{L}$  of the Stop Solution to each well, using a repeater or a multichannel pipet.
10. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. Please contact your plate manufacturer for details.
11. Use the plate reader's built-in 4PLC software capabilities to calculate RBP 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 should be multiplied by the dilution factor to obtain sample values. Sample RBP values should be normalized to creatinine levels by running the same samples in the Luminos Urinary Creatinine Detection Kit, K002-H1.

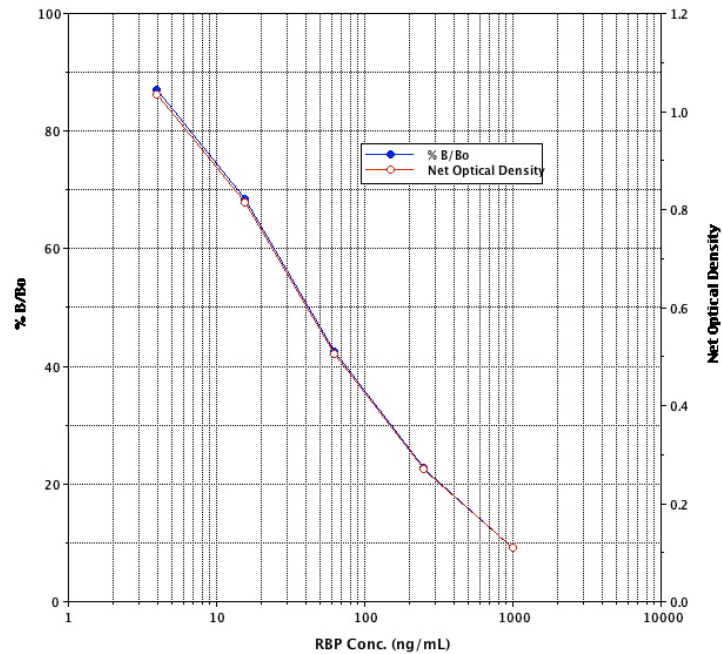
### Typical Data

Sample	Mean OD	Mean OD-NSB	%B/Bo	Concentration ng/mL
NSB	0.061	0	-	
Std 1	0.171	0.110	9.2	1000
Std 2	0.331	0.270	22.7	250
Std 3	0.567	0.506	42.5	62.5
Std 4	0.876	0.815	68.5	15.625
Std 5	1.096	1.035	87.0	3.906
Bo	1.251	1.19	100	0
Sample 1	0.289	0.228	19.1	321.6
Sample 2	0.739	0.678	57.0	29.27

**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 curve for calculation of results. Do not use this data.**

### Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the Bo and standard #5. The detection limit was determined at two (2) standard deviations from the Bo along the standard curve.

**Sensitivity was determined as 2.90 ng/mL.**

### Limit of Detection

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 urine sample.

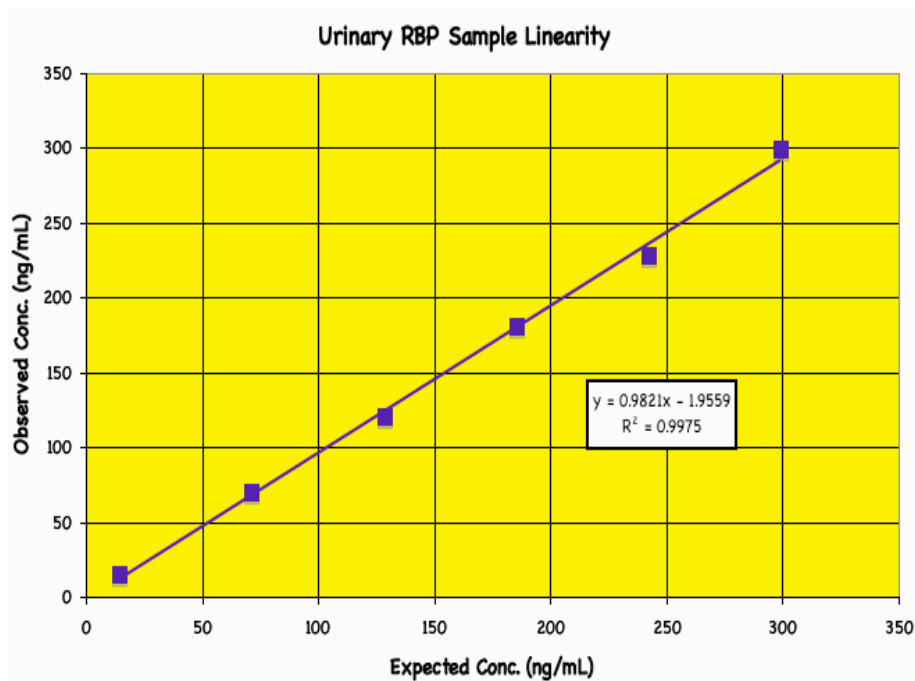
**Limit of Detection was determined as 4.09 ng/mL\*.**

\* 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
100%	0%	299.2	---	---
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
0%	100%	14.5	---	---
			<b>Mean Recovery</b>	<b>95.8%</b>



### Intra Assay Precision

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

Sample	RBP Conc. (ng/mL)	Standard Deviation	%CV
1	14.8	0.67	4.5
2	28.0	2.09	7.5
3	53.5	3.89	7.3
4	323.7	6.77	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 standard deviation of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	Standard Deviation	%CV
1	14.4	1.31	9.1
2	28.0	2.58	9.2
3	50.8	4.09	8.1
4	309.2	43.27	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 Luminos Urinary Creatinine Detection Kit (K002-H1) and the RBP levels normalized to creatinine levels. Normalized values ranged from 35.9 to 573.9  $\mu\text{g}$  RBP/g creatinine.

Normal ranges for urinary RBP is below 300 ng/mL.

## LIMITED WARRANTY

Luminos LLC 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:

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# PLATE LAYOUT

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

Printed on Forest Stewardship Council Certified paper

