



# Revised 14 Jan. 2013 rm (Vers. 2.1)



This kit is intended for Research Use Only.

# Not for use in diagnostic procedures.

## 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<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</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 hemo- statically controlled and does not fall until stores are dramatically reduced<sup>3-4</sup>.

RBP has been shown to be a useful surrogate marker for retinol because of the approximate 1:1 (molar) correlation between retinol and RBP in serum<sup>1</sup>-<sup>5-6</sup>, which implies that RBP may be used to assess and monitor vitamin A deficiency (VAD) in populations. The World Health Organization has estimated that 250 million children have moderate to severe VAD<sup>7</sup> due to lack of adequate nutrition, and the rising cost of food staples around the world further exacerbates this problem. In addition to nutritional deficiencies, infectious stresses have been shown to depress retinol concentrations. Therefore, individuals with diseases such as cystic fibrosis<sup>8</sup> and HIV-1<sup>9</sup> also run the risk of VAD due to the infectious stresses that contribute to the disease.

- Blaner WS. "Retinol binding protein: the serum transport protein for vitamin A." Endocr Rev. 1989 Aug;10(3):308-16.
- 2. Wolf G. "Multiple functions of vitamin A." Physiol. Rev. 1984 Jul;64(3):873-937.
- 3. Goodman DS, Blaner WS. in The Retinoids, eds. Sporn MB, Roberts AB, Goodman DS, (Orlando: Aca demic Press, 1984) vol.2, 1-39.
- 4. Olson, JA. "Vitamin A, retinoids and carotenoids." Modern Nutrition in Health and Disease, eds. Shils ME, Olson JA, Shike M, (Philadelphia: Lea & Febiger, 1994) 8th ed. 287-307.
- 5. Almekinder J, Manda W, Soko D, Lan Y, Hoover DR, Semba RD. "Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations." Scand J Clin Lab Invest. 2000 May;60(3):199-203.
- 6. Gamble MV, Ramakrishnan R, Palafox NA, Briand K, Berglund L, Blaner WS. "Retinol binding protein as a surrogate measure for serum retinol: studies in vitamin A-deficient children from the Republic of the Marshall Islands." Am J Clin Nutr. 2001 May;73(3):594-601.





# Revised 14 Jan. 2013 rm (Vers. 2.1)

- RUO
- 7. World Health Organization, Division of Nutrition, "Global Prevalence of Vitamin A Deficiency," Micro nutrient Deficiency Information System, No.2 (1995) Geneva: WHO/UNICEF.
- 8. Duggan C, Colin AA, Agil A, Higgins L, Rifai N. "Vitamin A status in acute exacerbations of cystic fibrosis." Am J Clin Nutr. 1996 Oct;64(4):635-9.
- 9. Baeten JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, Mandaliya K, Bwayo JJ, McClelland RS. "Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response." Am J Clin Nutr. 2004;79:218-25.

# ASSAY PRINCIPLE

The DRG Retinol Binding Protein (RBP) kit is designed to quantitatively measure RBP present in serum and plasma 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 450nm 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.

## REAGENTS

Retinol Binding Protein Tri-Level Controls for Low, Medium and High level human RBP in Serum For Quality Control and assay performance testing

# MATERIALS SUPPLIED

<ul> <li>Coated Clear 96 Well Plate</li> </ul>	One Plate				
A clear plastic microplate with break-apa	art strips coated with goat anti-rabbit IgG.				
RBP Standard	60 µL				
A stock solution of native human RBP at	t 100 μg/mL.				
RBP Antibody	3 mL				
A polyclonal antibody specific for RBP.					
<ul> <li>RBP-Peroxidase Conjugate</li> </ul>	5 mL				
An RBP-peroxidase conjugate.					
<ul> <li>Assay Buffer</li> </ul>	28 mL				
• Wash Buffer Concentrate	30 mL				
A 20X concentrate that should be diluted	d with deionized or distilled water.				
TMB Substrate	11 mL				
<ul> <li>Stop Solution</li> </ul>	11 mL				
A 1N hydrochloric acid solution. Causti	ic.				



# Revised 14 Jan. 2013 rm (Vers. 2.1)

• Plate Sealer

# **STORAGE INSTRUCTIONS**

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

# OTHER MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water.
- Repeater pipet with disposable tips capable of dispensing 25  $\mu$ L, 50  $\mu$ L and 100  $\mu$ L.
- A microplate shaker and washer.
- 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.

## 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 <u>all</u> 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 4.

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 serum and EDTA and heparin plasma samples only. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. They have been shown to yield artificially high RBP concentrations.

For detecting RBP in urine samples please refer to the Urinary Retinol Binding Protein Immunoassay kit, that is 10 times more sensitive than this serum kit.





1 each





# Revised 14 Jan. 2013 rm (Vers. 2.1)

RBP is a highly conserved protein and we have shown that the Urinary RBP kit, which uses identical antibody and conjugate, will measure RBP's from sources other than human including rat, dog and rhesus monkey. The end user should evaluate recoveries of RBP in other plasma and serum samples being tested.

### SAMPLE PREPARATION

Samples must be diluted 1:40 by taking one part of serum and adding thirty-nine parts of Assay Buffer prior to running in the kit. Any samples with RBP concentrations outside the standard curve range should be diluted further with Assay Buffer to obtain readings 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. 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.

#### 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 for 3 months at room temperature.

### **Standard Preparation**

Label seven glass test tubes #1 through #7. Briefly spin vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 475  $\mu$ L of Assay Buffer into tube #1 and 250  $\mu$ L into tubes #2-#7. Carefully add 25  $\mu$ L of the RBP Standard stock solution to tube #1 and vortex completely. Take 250  $\mu$ L of the RBP solution in tube #1 and add it to tube #2 and vortex completely. Add 250  $\mu$ L of tube #2 to tube #3 and vortex completely. Repeat these serial dilutions for tubes #4 through #7. The concentration of RBP in tubes 1 through 7 will be 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078  $\mu$ g/mL.

## Use all Standards within 2 hours of preparation.







# Revised 14 Jan. 2013 rm (Vers. 2.1)





	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	475	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	25	250	250	250	250	250	250
Final Conc (µg/mL)	5	2.5	1.25	0.625	0.3125	0.156	0.0781

# ASSAY PROTOCOL

- 1. Use the plate layout sheet on the back page 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 25 μL of samples or standards into wells in the plate. Pipet 50 μL of Assay Buffer into the non-specific binding (NSB) wells. Pipet 25 μL of Assay Buffer into wells to act as maximum binding wells (B0).
- 3. Add 50 µL of the RBP-peroxidase conjugate to each well, using a repeater pipet.
- 4. Add 25 µL of the RBP Antibody solution 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  $\mu$ L wash buffer. Tap the plate dry on clean absorbent towels.
- 7. Add 100  $\mu$ L of the TMB Substrate to each well, using a repeater pipet.
- 8. Incubate the plate at room temperature for 15 minutes.
- 9. Add 100  $\mu$ L 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.

# **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, after subtracting the mean OD's for the blank. The sample concentrations, calculated off the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

DRG International, Inc., USA Fax: (973) 564-7556 e-mail: corp@drg-international.com 5





# Revised 14 Jan. 2013 rm (Vers. 2.1)

RUO	
-----	--

Sample	Mean OD	Net OD	% B/B0	RBP Conc. (µg/mL)
NSB	0.145	0	-	-
Standard 1	0.538	0.393	12.0	5
Standard 2	0.816	0.671	20.6	2.5
Standard 3	1.074	0.929	28.5	1.25
Standard 4	1.594	1.449	44.4	0.625
Standard 5	2.025	1.881	57.6	0.313
Standard 6	2.496	2.351	72.0	0.156
Standard 7	2.830	2.686	82.3	0.078
B0	3.409	3.264	100.0	0
Sample 1	1.666	1.521	46.6	0.538
Sample 2	1.066	0.921	28.2	1.376

#### **TYPICAL DATA**

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

Conversion Factor: 1 µg/mL of human RBP is equivalent to 47.62 nM RBP

# **Typical Standard Curve**



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

**DRG International, Inc., USA** 

Fax: (973) 564-7556

e-mail: corp@drg-international.com 6





# Revised 14 Jan. 2013 rm (Vers. 2.1)



# **REFERENCES** (cont'd.)

- 10. ARUP's Laboratory Test Directory. "Retinol Binding Protein." 2008. Internet: <u>http://www</u>. aruplab.com/guides/ug/tests/0050467.jsp (23 May 2008).
- 11. Hix J, Martinez C, Buchanan I, Morgan J, Tam M, Shankar A. "Development of a rapid enzyme immunoassay for the detection of retinol-binding protein." Am J Clin Nutr 2004;79:93-8.
- 12. Tietz, N.W., in "Textbook of Clinical Chemistry", WB Saunders, 1986.

## LIMITED WARRANTY

DRG International 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.



Revised 14 Jan. 2013 rm (Vers. 2.1)

т	G	п	т	٥	c	œ	Þ	$\square$
								-
								2
								ы
			÷					4
								S
								თ
								7
								œ
								9
					~~~			10
								=
								12

Version 02-Nov-12\_rm

DRG International, Inc., USA Fax: (973) 564-7556 e-mail: corp@drg-international.com

RUO

8