DRG® Urinary Retinol Binding Protein ELISA (EIA-5205)

As of 10 May 2012 rm (Vers. 1.1) **RUO**

Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The DetectX® Urinary Retinol Binding Protein (RBP) kit is designed to 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 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.

MATERIALS SUPPLIED

- **Coated Clear 96 Well Plate** **One Plate**
  A clear plastic microplate with break-apart strips coated with goat anti-rabbit IgG.
- **RBP Standard** **60 µL**
  A stock solution of native human RBP at 20 Mg/mL.
- **DetectX® RBP Antibody** **3 mL**
  A polyclonal antibody specific for RBP.
- **DetectX® RBP-Peroxidase Conjugate** **3mL**
  A RBP-peroxidase conjugate.
- **Assay Buffer** **28 mL**
- **Wash Buffer Concentrate** **30 mL**
  A 20X concentrate that should be diluted with deionized or distilled water.
- **TMB Substrate** **11 mL**
- **Stop Solution** **11 mL**
  A 1N hydrochloric acid solution. **Caustic.**
- **Plate Sealer** **1 each**

STORAGE INSTRUCTIONS

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

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OTHER MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water.
- A microplate shaker and a microplate washer.
- 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.

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 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 fully validated for human urine samples and tested in rat, dog and rhesus monkey urines. 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'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, please refer to the Serum RBP Immunoassay kit, EIA-5202.

SAMPLE PREPARATION

Samples must be diluted 1:2 by adding one part of urine to one part 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.

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REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. 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.

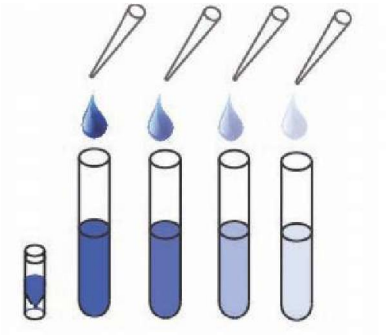
WASH BUFFER PREPARATION

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

STANDARD PREPARATION

Label five glass test 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.



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

ASSAY PROTOCOL

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 wells to act as maximum binding wells (Bo).
3. Add 25 µL of the DetectX® RBP-peroxidase conjugate to each well, using a repeater pipet.
4. Add 25 µL of the DetectX® 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 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 100 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.

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.

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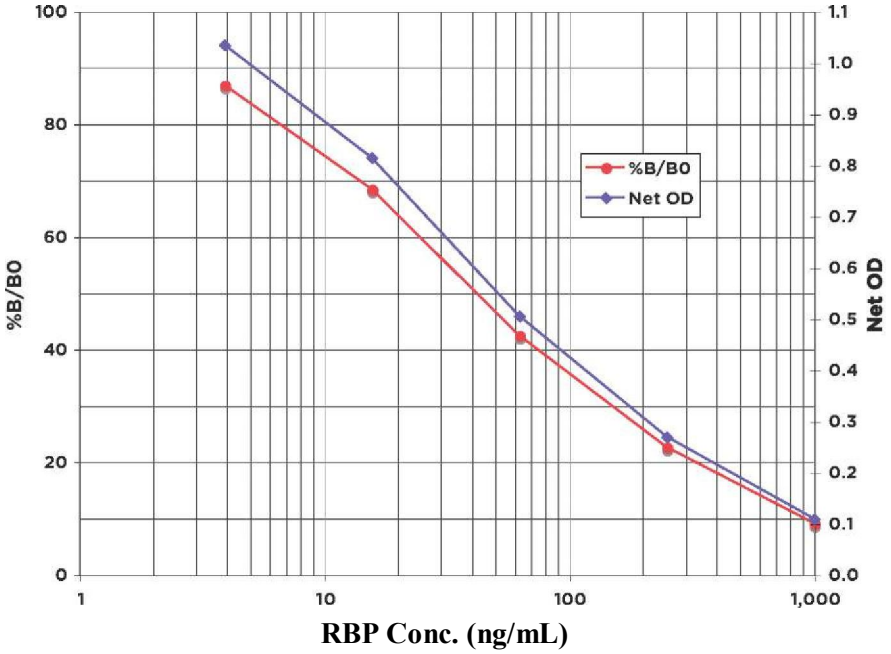
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	RBP Conc. (ng/mL)
NSB	0.061	0	-	-
Standard 1	0.171	0.110	9.2	1,000
Standard 2	0.331	0.270	22.7	250
Standard 3	0.567	0.506	42.5	62.5
Standard 4	0.876	0.815	68.5	15.625
Standard 5	1.096	1.035	87.0	3.906
B0	1.251	1.190	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



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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.

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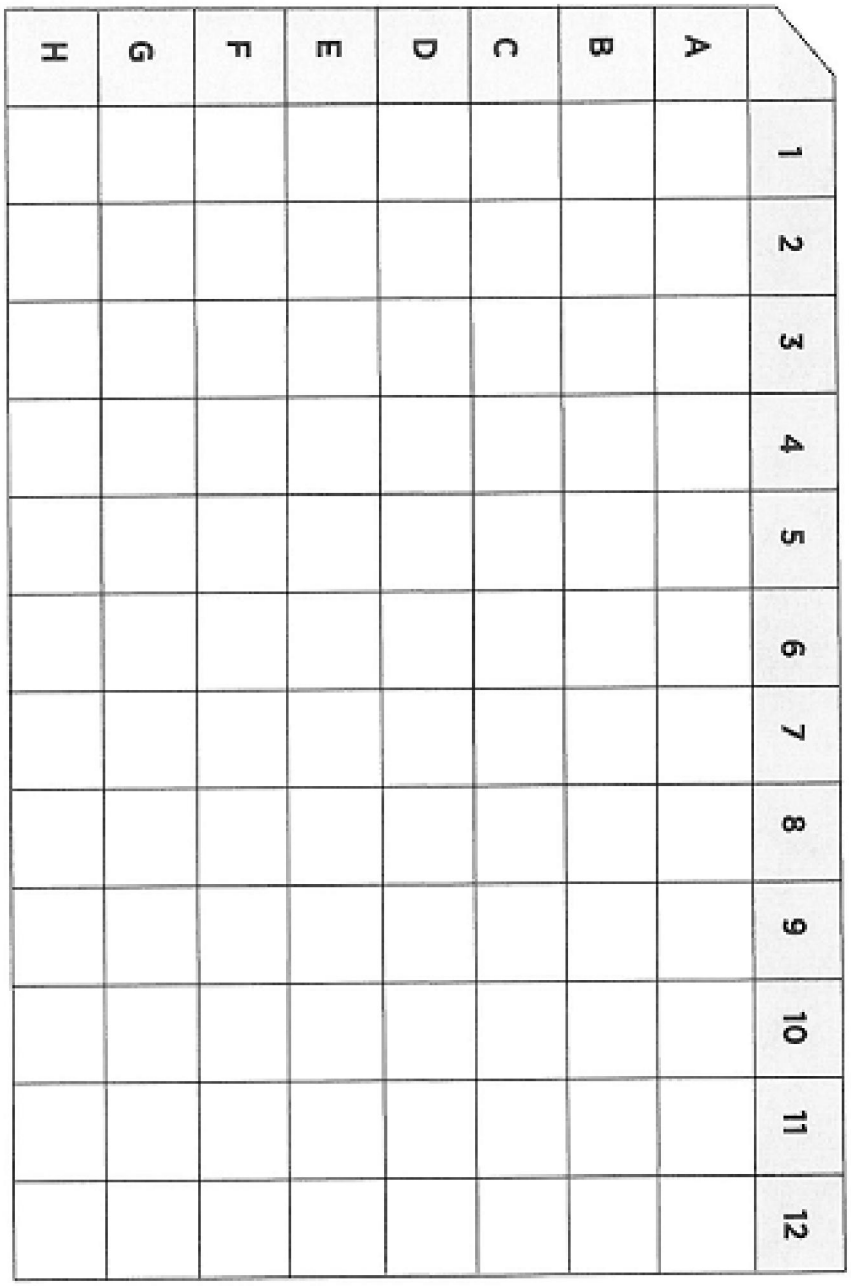

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