StressMarq Biosciences INC.

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StressXpress®

Retinol Binding Protein Urinary EIA Kit

Catalog# SKT-218 (96-Well Kit)

Colorimetric measurement of RBP levels in urine

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-218A	Coated Clear 96 Well Plate	One Plate	A clear plastic microplate with break-apart strips coated with goat anti-rabbit IgG.
SKC-218B	RBP Standard	60 µL	A stock solution of native human RBP at 20 µg/mL
SKC-218C	StressXpress® RBP Antibody	3 mL	A polyclonal antibody specific for RBP
SKC-218D	StressXpress® RBP-Peroxidase Conjugate	3 mL	A RBP-peroxidase conjugate
SKC-218E	Assay Buffer	28 mL	
SKC-218F	Wash Buffer Concentrate	30 mL	A 20X concentrate that should be diluted with deionized or distilled water
SKC-218G	TMB Substrate	11 mL	-
SKC-218H	Stop Solution	11 mL	-
SKC-218I	Plate Sealer	1 each	-

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

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 and 9.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Storage

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

Materials Needed 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.

Please read this booklet completely prior to using the product.

INTRODUCTION

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).

Assay Overview

The Retinol Binding Protein Urinary StressXpress® EIA 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 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.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Human, Rat, Dog and Rhesus Monkey Urine

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. Please see page 16 for details of other urine samples tested. The end user should evaluate recoveries of RBP in other urine samples being tested.

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.

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.

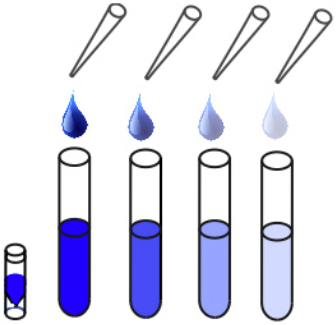
Wash Buffer Preparation

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

Standard Preparation

Label five 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.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Assay Buffer Volume (µL)	475	300	300	300	300
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 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

1. Use the plate layout sheet on page 19 of the kit booklet 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 StressXpress® RBP-peroxidase conjugate to each well, using a repeater pipet.

4. Add 25 µL of the StressXpress® 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. nm.

ANALYSIS

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. Sample RBP values should be normalized to creatinine levels by running the same samples in the StressXpress® Creatinine Urinary Detection Kit, SKT-200

Typical Data

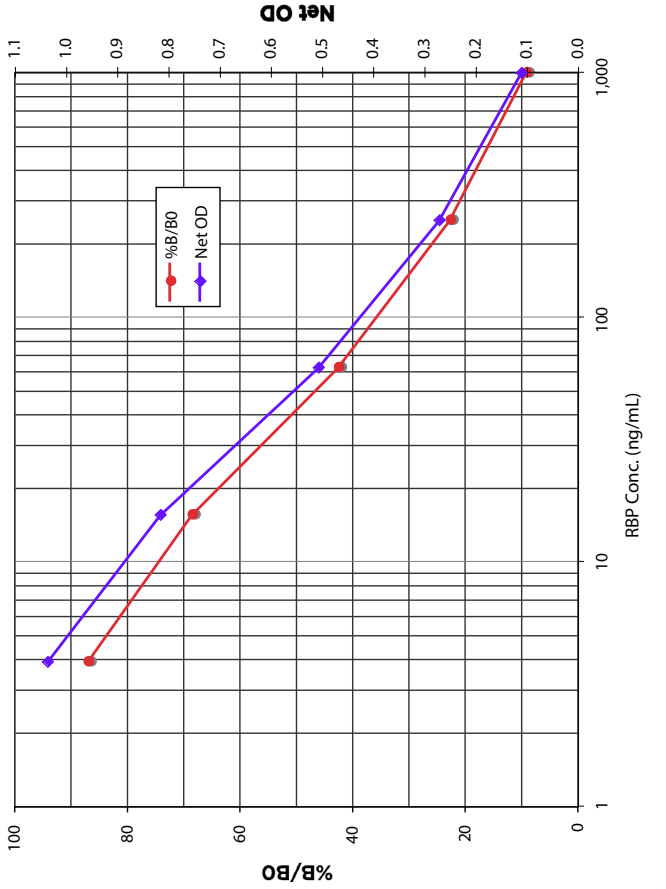
Sample	Mean OD	Net OD	% B/B0	RBP Concentration. (ng/mL)
NSB	0.061	0	-	-
Standard 1	0.171	0.110	9.2	1,000
Standard 2	0.331	0.270	22.7	250
Standard 3	0.567	0.506	42.5	62.5
Standard 4	0.876	0.815	68.5	15.625
Standard 5	1.096	1.035	87.0	3.906
B0	1.251	1.190	100	0
Sample 1	0.289	0.228	19.1	321.6
Sample 2	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

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.

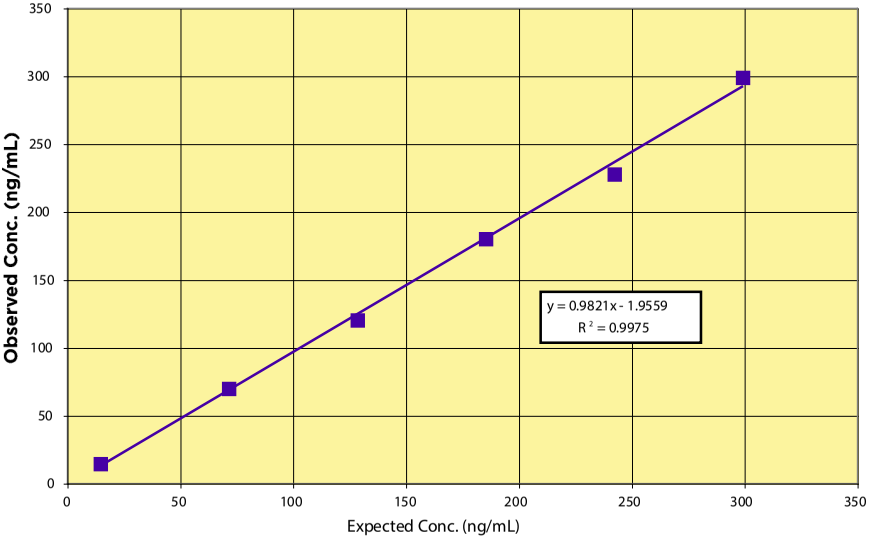
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration human urine sample.
Limit of Detection was determined as 4.09 ng/mL*.

* 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
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	---
			Mean Recovery	**95.8%**


Observed Conc. (ng/mL)
Expected Conc. (ng/mL)
y = 0.9821x - 1.9559
R² = 0.9975
0
50
100
150
200
250
300
350

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 StressXpress® Creatinine Urinary Detection Kit, SKT-200, 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 5015.

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.

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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Technical Service Contact Information

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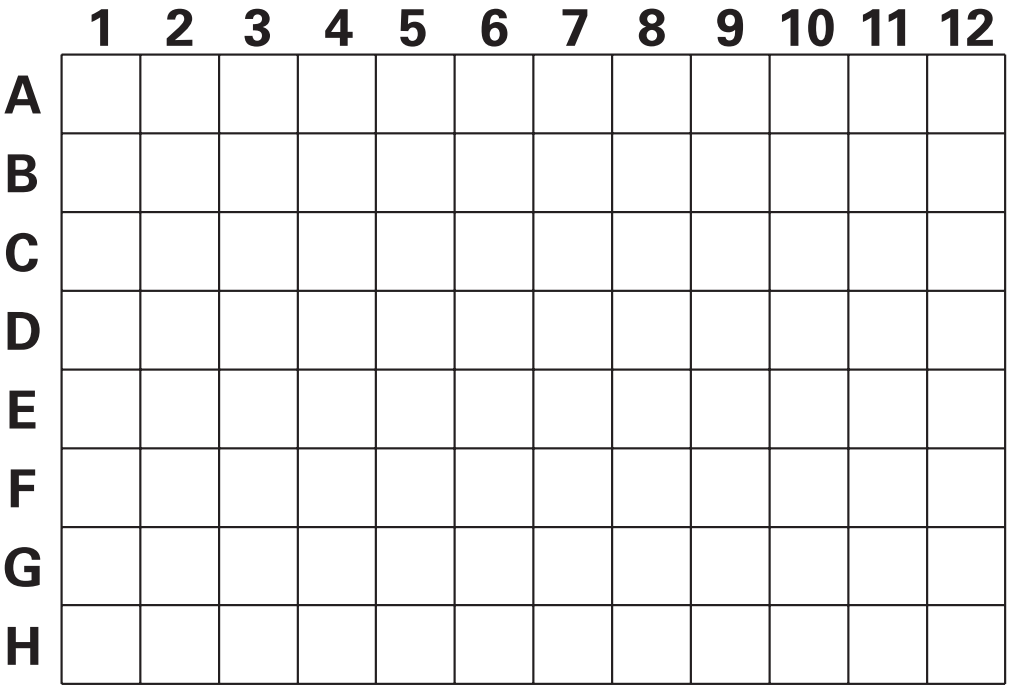
Fax:	250-294-9025
E-Mail:	techsupport@stressmarq.com
Hours:	M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).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