ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Retinol Binding Protein
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K062-H1
5 Plate Kit Catalog Number K062-H5

Species Independent

Multi-Format Kit for Multiple Samples Types

Sample Types Validated:

**Serum, EDTA and Heparin Plasma,
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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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 been shown to be a useful surrogate marker for retinol because of the approximate 1:1 (molar) correlation between retinol and RBP in serum[1, 6,7], 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[7] 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.

RBP has also been shown to be a useful marker for renal function[8] as it is totally filtered by the glomeruli and reabsorbed by proximal tubules[9]. This has made the measurement of urinary RBP a tool to study renal function in heart[10] or kidney[11] transplant recipients.

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6. Almekinder, J., et al. (2000). Evaluation of plasma retinol-binding protein as a surrogate measure for plasma retinol concentrations. *Scandinavian Journal of Clinical and Laboratory Investigation, 60*(3), 199–203.
7. Gamble, M.V., et al. (2001). Retinol binding protein as a surrogate measure for serum retinol: Studies in vitamin A-deficient children from the Republic of the Marshall Islands. *American Journal of Clinical Nutrition, 73*(3), 594–601.
8. Peterson, P.A. & Berggård, I. (1971). Isolation and properties of a human retinol-transporting protein. *Journal of Bioligical Chemistry, 246*, 25–33.
9. Bernard, A.M. & Lauwerys, R.R. (1981) Retinol-binding protein in urine: A more practical index than urinary ß2-microglobulin for the screening of renal tubular function. *Clinical Chemistry, 27*, 1781–1782.
10. Camara, N.O., et al. (2001). Early detection of heart transplant patients with increased risk of cyclosporin nephrotoxicity. *The Lancet, 357*, 856-857.
11. Hosaka, B., et al. (2003). Predictive value of urinary retinol-binding protein for graft dysfunction after kidney transplantation. *Transplantation Proceedings, 35*, 1341–1343.

ASSAY PRINCIPLE

The DetectX® Multi-Format Retinol Binding Protein (RBP) kit is designed to quantitatively measure RBP present in serum, plasma, dried blood spot (DBS) and urine samples. Please read the complete kit insert before performing this assay.

The kit offers two standard curve ranges. For serum and plasma samples, we recommend using 10 µL of standards and samples with an assay range of 1,000 to 7.813 ng/mL. For urine and dried blood spot samples, we recommend using 100 µL of standards and samples with an assay range of 200 to 1.563 ng/mL.

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 sheep 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 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
Hemoglobin Detection Kit (2 Plate)	K013-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 donkey anti-sheep IgG.

Kit K062-H1 **or** -H5	1 **or** 5 Each	Catalog Number X061-1EA

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

Kit K062-H1 **or** -H5 6	0 µL **or** 240 µL	Catalog Number C014-60UL **or** -240UL

DetectX® RBP Antibody
A polyclonal antibody specific for RBP.

Kit K062-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C230-3ML **or** -13ML

DetectX® RBP Conjugate
A RBP-peroxidase conjugate.

Kit K062-H1 **or** -H5	3 mL **or** 13 mL	Catalog Number C015-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that must be diluted with deionized or distilled water.

Kit K062-H1 **or** -H5 2	8 mL **or** 55 mL	Catalog Number X053-28ML **or** -55ML

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

Kit K062-H1 **or** -H5	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K062-H1 **or** -H5	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

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

Kit K062-H1 **or** -H5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K062-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 serum, plasma, 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 samples tested. The end user should evaluate recoveries of RBP in other samples being tested.

SAMPLE PREPARATION

Serum and Plasma

10 µL Format (See page 9) Serum and plasma samples must be diluted 1:40 by taking one part of serum or plasma and adding thirty-nine parts of diluted Assay Buffer (see page 8) prior to running in the kit.

Urine Samples

100 µL Format (See Page 9) Urine samples must be diluted 1:4 by adding one part of urine to three parts 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.

Dried Blood Spots (DBS)

100 µL Format (See Page 9) 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. The DBS samples require ≥ 1:60 dilution in diluted Assay Buffer.

One 1/4" DBS sample is equivalent to 6 µL of a whole blood sample and we recommend adding 900 µL diluted Assay Buffer. This is a dilution of 1:150. Two 1/8" DBS will contain the equivalent to 3 µL of whole blood sample and we recommend adding 900 µL diluted Assay Buffer. This is a dilution of 1:300. 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 1/4 inch DBS is considered to contain 6 µL of whole blood and 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 shortly after 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

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.

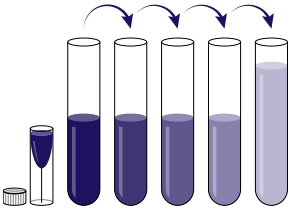
Wash Buffer

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

Standard Preparation - 10 µL Assay Format - Serum and Plasma Samples

Label test tubes as #1 through #8. Pipet 190 µL of Assay Buffer into tube #1 and 50 µL into tubes #2 to #8. Carefully add 10 µL of the RBP stock solution to tube #1 and vortex completely. Take 50 µL of the RBP solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of RBP in tubes 1 through 8 will be 1,000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.813 ng/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**190**	50	50	50	50	50	50	50
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	50	50	50	50	50	50	50
Final Conc (ng/mL)	1,000	500	250	125	62.5	31.25	15.625	7.813

Standard Preparation - 100 µL Assay Format - Urine and Dried Blood Spot Samples

Label test tubes as #1 through #8. Pipet 990 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #8. Carefully add 10 µL of the RBP stock solution to tube #1 and vortex completely. Take 300 µL of the RBP solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of RBP in tubes 1 through 8 will be 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.563 ng/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**990**	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	300	300	300	300	300	300	300
Final Conc (ng/mL)	200	100	50	25	12.5	6.25	3.125	1.563

Use all Standards within 2 hours of preparation.

ASSAY PROTOCOL - 10 µL AND 100 µL

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 to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 10 µL (100 µL for alternate format) of samples or standards into wells in the plate.
3. Pipet 35 µL (125 µL for alternate format) of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 10 µL (100 µL for alternate format) of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® RBP Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® RBP Antibody to each well, except the NSB wells, using a repeater pipet.
7. 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. If the plate is not shaken signals bound will be approximately 15% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. 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. Urinary sample RBP values should be normalized to creatinine levels by running the same samples 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

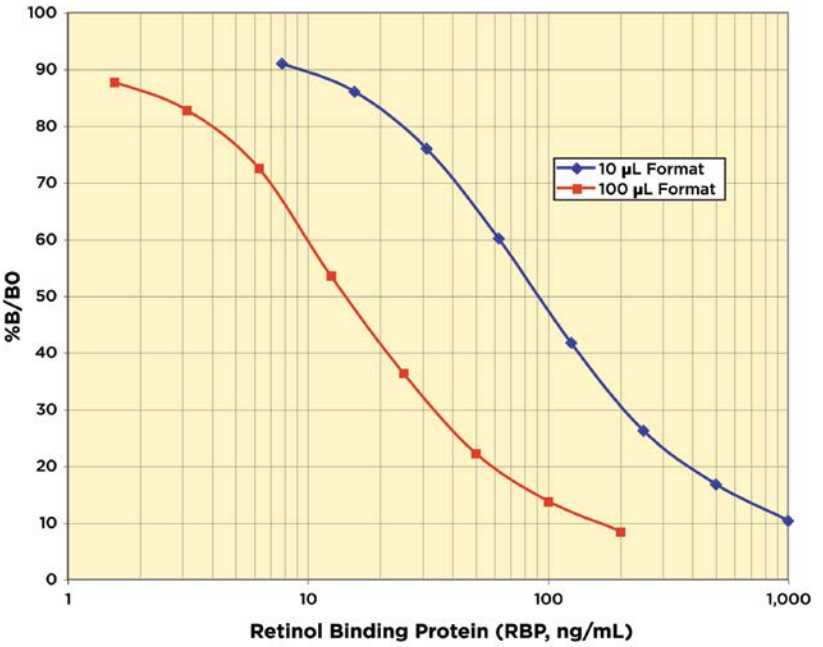
TYPICAL DATA

	10 µL Assay				100 µL Assay			
Sample	**Mean OD**	**Net OD**	**% B/B0**	**RBP Conc. (ng/mL)**	**Mean OD**	**Net OD**	**% B/B0**	**RBP Conc. (ng/mL)**
NSB	0.066	0.000	-	-	0.064	0.000		-
Standard 1	0.234	0.168	10.3	1,000	0.137	0.073	8.64	200
Standard 2	0.338	0.272	16.7	500	0.182	0.118	14.0	100
Standard 3	0.492	0.426	26.2	250	0.254	0.190	22.5	50
Standard 4	0.744	0.678	41.7	125	0.373	0.309	36.6	25
Standard 5	1.043	0.977	60.2	62.5	0.518	0.454	53.7	12.5
Standard 6	1.300	1.234	76.0	31.25	0.678	0.614	72.7	6.25
Standard 7	1.463	1.397	86.0	15.625	0.764	0.700	82.8	3.125
Standard 8	1.544	1.478	91.0	7.813	0.806	0.742	87.8	1.563
B0	1.690	1.624	100	0	0.909	0.845	100	0
Sample 1	0.778	0.712	43.8	116.2	0.337	0.273	32.3	29.7
Sample 2	1.131	1.065	65.6	50.45	0.580	0.516	61.1	7.22

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 with the 10 µL and the 100 µL sample volume was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the appropriate volume standard curve.

Sensitivity was determined as 5.69 ng/mL for 10 µL and 1.36 ng/mL for 100 µL sample size.

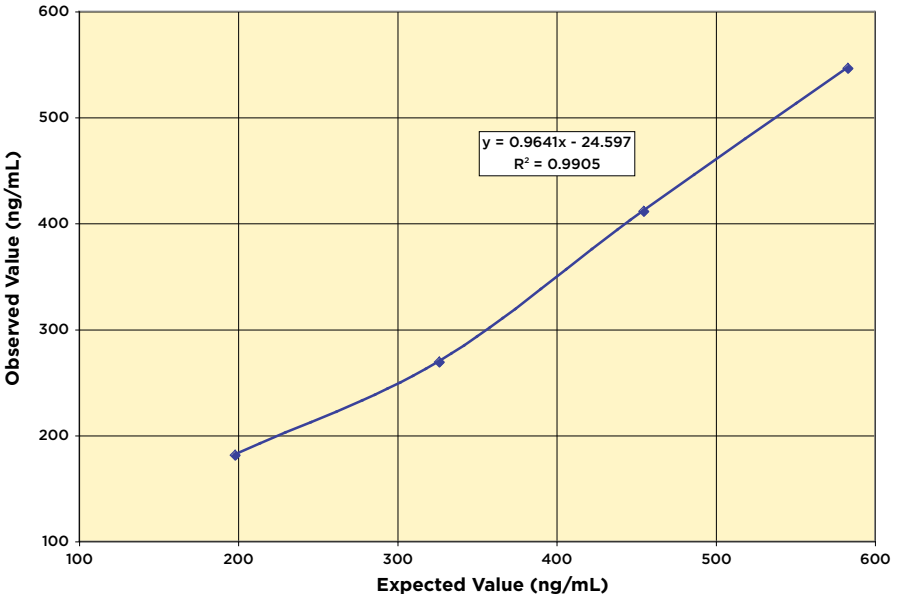
The Limit of Detection for the 10 µL and the 100 µL sample format was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample in the appropriate volume standard curve.

Limit of Detection was determined as 8.09 ng/mL for 10 µL and 0.995 ng/mL for 100 µL sample size.

Linearity

Linearity was determined for the 10 µL format using human plasma samples, by taking samples with a high known RBP concentration and a lower RBP concentration and mixing them in the ratios given below. The measured RBP concentrations were compared to the expected values based on the ratios used.

High Sample	Low sample	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	583.1	546.2	93.7%
60%	40%	454.8	411.4	90.5%
40%	60%	326.4	269.2	82.5%
20%	80%	198.1	181.1	91.4%
			Mean Recovery	**89.5%**



Intra Assay Precision - 10 µL Format

Three human serum and plasma samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	%CV
1	52.6	6.1
2	120.8	4.8
3	182.6	3.7

Inter Assay Precision - 10 µL Format

Three human serum and plasma samples were diluted with Assay Buffer and run in duplicates in twenty-three assays run over multiple days by four operators. The mean and precision of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	%CV
1	45.3	16.0
2	112.0	11.6
3	174.6	9.3

Inter Assay Precision - 100 µL Format

Three human serum and plasma samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by four operators. The mean and precision of the calculated RBP concentrations were:

Sample	RBP Conc. (ng/mL)	%CV
1	8.2	11.4
2	19.9	9.3
3	31.5	9.9

SAMPLE VALUES

Eleven random human urine samples were tested in the assay. Values adjusted for dilution ranged from 7.13 to 98.66 ng/mL with a mean of 32.27 ng/mL.

Ten normal human serum samples were tested in the assay. Values adjusted for dilution ranged from 23.1 to 45.4 µg/mL with a mean of 32.38 µg/mL. Eleven normal human plasma samples were tested in the assay. Adjusted values ranged from 18.13 to 49.8 µg/mL with a mean of 26.70 µg/mL.

Twenty random human whole blood DBS samples were punched out as 1/4" or 1/8" and tested in the assay. Adjusted values ranged from 9.80 to 33.67 µg/mL with an average of 18.8 µg/mL.

CROSS REACTIVITY

The following cross reactant was tested in the assay and calculated at the 50% binding point.

Analyte	Cross Reactivity (%)
RBP4	23.2%

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

For details concerning this kit or to order any of our products please contact us:

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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.

ASSAYS™
FOR SERIOUS SCIENCE

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