Retinol Binding Protein (RBP) Urinary Competitive ELISA Kit

Catalog Number EIARBPUR (96 tests), EIARBPURX10 (10 x 96 tests)

Rev 2.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Retinol Binding Protein (RBP) Urinary Competitive ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of retinol binding protein (RBP) in 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 this kit has been shown to measure RBP from sources other than human. See page 4 for further information. Evaluate recoveries of RBP in other urine samples being tested.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity (96 tests)	Quantity (10 x 96 tests)
RBP Standard; 20 µg/mL RBP in a special stabilizing solution	60 µL	10 x 60 µL
Assay Buffer Concentrate (5X)	28 mL	10 x 28 mL
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate	10 plates
RBP Antibody	3 mL	10 x 3 mL
RBP Conjugate	3 mL	10 x 3 mL
Wash Buffer Concentrate (20X)	30 mL	2 x 125 mL
TMB (Tetramethylbenzidine) Substrate	11 mL	10 x 11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL	1 x 50 mL
Plate Sealer	1	10

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Plate shaker
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge, or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

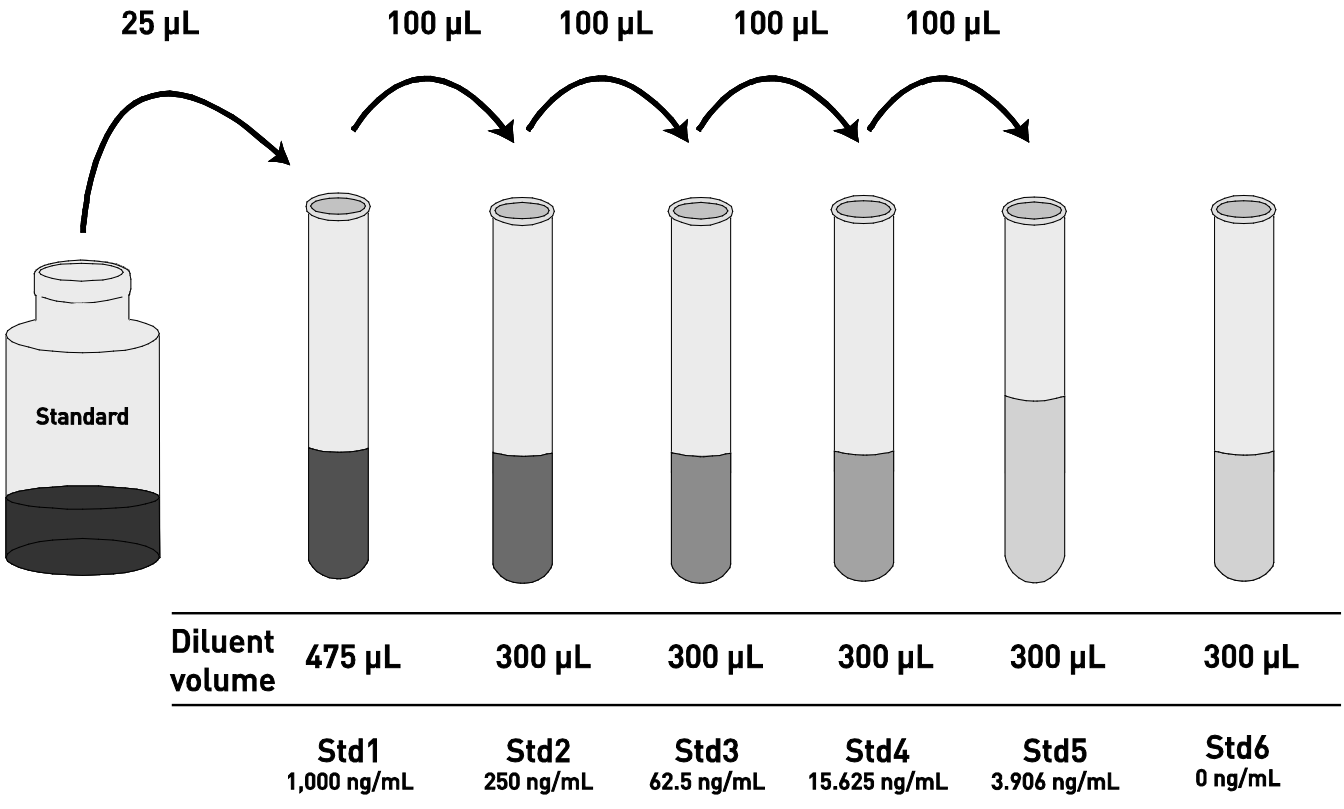
Sample type	Procedure
Urine	Dilute samples 1:2 by adding one part of urine to one-part 1X Assay Buffer prior to running in the kit. Dilute any samples with RBP concentrations greater than the standard curve range with 1X Assay Buffer to obtain readings within the standard curve. Samples that are too dilute to be measured should be concentrated prior to measuring in the assay.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 1,000 to 3.906 ng/mL, but a curve can be obtained using a range of 250 to 3.906 ng/mL. Choose the range that fits your sample concentrations most appropriately.

1. Add 25 µL RBP Standard to one tube containing 475 µL 1X Assay Buffer and label as 1,000 ng/mL RBP.
2. Add 100 µL 1X Assay Buffer to each of 5 tubes labeled as follows: 250, 62.5, 15.625, 3.906, and 0 ng/mL RBP.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

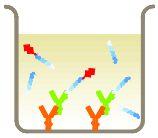


Perform ELISA (Total assay time: 1.5 hours)

IMPORTANT! Perform a standard curve with each assay.

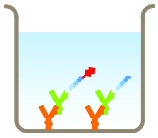
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



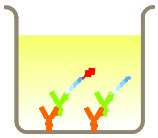
Bind antigen

a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of RBP Conjugate to each well.
d. Add 25 µL of RBP Antibody to each well except NSB wells.
e. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 1 hour at room temperature with shaking.
   Note: If the plate is not shaken the bound of the signals will be ~45% lower.
f. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.
   Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns, and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals lower than that of the highest standard and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–1,000 ng/mL retinol binding protein (RBP).

Standard Retinol Binding Protein (RBP) (ng/mL)	Optical Density (450 nm)*
1,000	0.194
250	0.319
62.5	0.516
15.625	0.740
3.906	0.907
0	0.999

Note: The NSB gave a Mean OD value of 0.098.

Intra-assay precision

Samples were assayed in replicates of 8 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (ng/mL)	14.8	28.0	53.5	323.7
%CV	4.5	7.5	7.3	2.1

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 21 assay runs by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Mean (ng/mL)	14.4	28.0	50.8	309.2
%CV	9.1	9.2	8.1	14

CV = Coefficient of Variation

Performance characteristics, continued

Expected 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 Urinary Creatinine Detection Kit, EIACUN, 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 50.

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.

Recovery

Recovery 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 Sample %	Low Sample %	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
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
			Mean Recovery	**95.8%**

Sensitivity

The analytical sensitivity of RBP is 2.90 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288