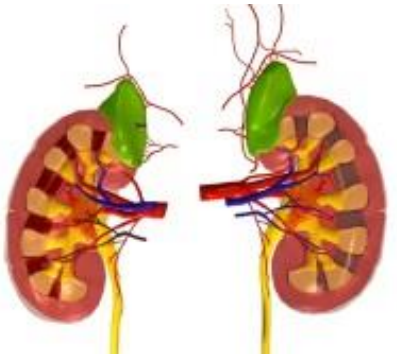
BioAssay Works®

Rat Kim-1 ELISA Test Kit

For the Detection of Kim-1 in Rat Urine

For Research Use Only

Catalog Number: R-RENA-E-001



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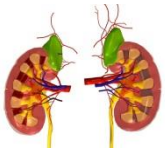

Rat Kim-1 ELISA TEST KIT for the Detection of Kim-1 in Rat Urine

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Cat. #: R-RENA-E-001

Intended Use

The Bioassay Works, Inc. Rat Kim-1 ELISA test kit is intended for use in the detection and quantitation of Kidney Injury Molecule-1 (Kim-1) in rat urine.

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Summary and Explanation

Kim-1 is a type I trans-membrane structural glycoprotein located in the renal proximal tubule epithelial cells. These cells undergo regeneration after various forms of injury and shed Kim-1 antigen into the urine. Thus, urinary Kim-1 is an early and specific biomarker for tubular kidney injury. Kim-1 has become widely recognized as an excellent tool in pre-clinical studies to monitor acute kidney tubular toxicity.

Kidney injury caused by therapeutic agents and drug induction is a common type of injury requiring appropriate monitoring and intervention. Current standards using blood urea nitrogen and creatinine are considered late indicators of kidney injury and are often non-specific. Kim-1 has consistently outperformed traditional biomarkers of kidney injury in preclinical biomarker studies[1]. The detection of Kim-1 can occur in as little as six hours post injection of an agent known to cause kidney injury[2].

Test Principle

The Rat Kim-1 ELISA Test Kit is an antigen capture assay to quantitatively determine the concentration of kidney injury molecule-1 (Kim-1) in urine specimens. Serially diluted Kim-1 calibrator and diluted urine specimens are added to the anti-Kim-1 MAb solution where Kim-1, if present in the specimen, binds to the anti-Kim-1 antibody. The antigen-antibody complex then binds to the anti-Kim-1 antibody immobilized on the microtiter plate/strip. Streptavidin horseradish peroxidase is added followed by the addition of ABTS substrate to reveal the presence of rat Kim-1 by the enzymatic conversion of the colorless substrate to green. The color change is evaluated by measuring the optical density (O.D.) at 405 nm with a reference filter measurement at 490 nm using a spectrophotometric plate or strip reader. The optical densities of the calibrator dilutions are graphed, and the concentration of specimens projected from the linear range of the standard curve.

Thorough washing is required prior to the addition of each reagent.

The assay provides reliable results in the detection of rat Kim-1 at concentrations $\geq$ 80 pg/mL.

Materials Provided

Each Kit contains sufficient material for 96 tests.

1. Rat Kim-1 antibody coated plate, Cat. No. R-RENA-E-002. One 12 x 8well plate of microwell strips coated with anti-Rat Kim-1 antibody in a resealable pouch with desiccant.
2. Rat Kim-1 Calibrator, Cat. No. RKC-001. Two vials containing lyophilized recombinant Rat Kim-1 in a buffered protein based stabilizer - 40 ng/mL after reconstitution.
3. Reconstitution Solution, Cat. No. RS-002. One vial containing 1.8 mL of laboratory grade water with preservative.
4. KIM-1 Negative Control, Cat. No. HKNC-001. One vial containing 200µL of a buffered protein solution.
5. Sample Dilution Buffer, Cat. No. KSDB-012. One bottle containing 12 mL of phosphate buffered saline, protein stabilizer, detergent, preservative and **yellow dye**.
6. Rat KIM-1 MAb Solution, Cat. No. RKMS-006. One bottle containing 6 mL of anti-Rat Kim-1 monoclonal antibody conjugated to biotin in phosphate buffered saline, protein stabilizer, detergent, preservative and **blue dye**.
7. Peroxidase Conjugate Solution, Cat. No. PCS-012. One bottle containing 12 mL of horseradish peroxidase conjugated streptavidin in a buffered protein solution, enzyme stabilizers and preservative.
8. 20x Wash Solution, Cat. No. KWS-050. One bottle containing 50 mL of 20x phosphate buffered saline, detergent and preservative.
9. ABTS Substrate Solution, Cat. No. KABTS-012. One bottle containing 12 mL ABTS Substrate Solution – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt.
10. Stop Solution, Cat. No. KSS-012. One bottle containing 12 mL of 1% Lauryl Sulfate with preservative.

Equipment and Materials Required but Not Provided

- Laboratory quality distilled/deionized water
- Waterbath or incubator capable of maintaining humid conditions at 37°C (± 2°C)
- 96-well microtiter plate(s), low protein binding, for performing primary dilutions
- Pipettor and disposable tips capable of delivering 25–150 µL
- EIA Plate Reader capable of reading optical density at 405 nm (dual wavelength reference filter 490 nm)
- Plate washing apparatus

Warnings and Precautions

- Treat all clinical specimens and any material coming into contact with them as potentially infectious. Wear disposable gloves when handling specimens and kit components.
- The ABTS Substrate Solution has been reported to be non-carcinogenic but contact with skin and mucous membranes should be avoided. Rinse with copious amounts of water in the event of exposure.

- Dispose of clinical material and potentially infectious material in accordance with local regulations.
- Do not mix components of one lot of kits with components from other lots.
- Do not use kit components beyond their expiration dates.
- Do not use reagents that show signs of contamination.
- Good Laboratory Practices should be employed to avoid cross contamination of specimens and reagents.

Specimen Collection

Aseptically collect urine in a sterile container using standard good laboratory practices. Centrifuge to remove particulates and assay immediately or aliquot and store frozen.

Stability and Storage

When stored at 2-8°C, the Rat Kim-1 ELISA Kit is stable up to the expiration date printed on the kit label.

Do not freeze or expose to elevated temperatures**.**

Discard any remaining reagents after the expiration period.

Reagent Preparation

Allow the kit to warm to room temperature (20-25°C) prior to use.

Warm the 20x Wash Solution, Cat. No. KWS-050, and the Stop Solution, Cat. No. KSS-012, to re-dissolve any salts that may have formed during refrigerated storage.

Prepare working strength Wash Solution by adding 1 part 20x Wash Solution to 19 parts laboratory grade water (1/20 dilution). It is recommended that working strength Wash Solution be prepared as required on the day of use. Remaining 20x Wash Solution should be stored at 2-8°C.

Reconstitute the Rat Kim-1 Calibrator, Cat. No. RKC-001, by adding 0.6 mL of the Reconstitution Solution, Cat. No. RS-002, directly into the vial. Mix **gently** and allow to sit for 15 minutes. Mix thoroughly but gently prior to use.

Procedure

Primary calibrator dilution

Perform a primary 2-fold serial dilution of the Rat Kim-1 Calibrator, Cat. No. RKC-001, in 8 wells of a low binding microtiter plate/strip or in 8 x 1.5 mL microtubes as follows:

- Add 150 µL Sample Dilution Buffer, Cat. No. KSDB-012, **yellow dye**, to all 8 wells/tubes.
- Add 150 µL of the reconstituted Rat Kim-1 Calibrator, prepared previously, to the first well/tube. Mix well and transfer 150 µL to the second well/tube. Mix well and continue the 2-fold dilution throughout the range of the titration.
- The 2-fold serial dilution yields Rat Kim-1 concentrations of 20 ng/mL, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 ng/mL.

Primary urine specimen dilution

Perform a primary 1/3 dilution of urine specimens in the wells of a low binding microtiter plate/strip or in 1.5 mL microtubes as follows:

- Add 100 µl of Sample Dilution Buffer, Cat. No. KSDB-012, **yellow dye**, to all required wells/tubes.
- Add 50 µL of each test specimen to the designated well/tube and mix thoroughly.

A secondary dilution of the calibrators and specimens is performed in the ELISA plate during testing, reducing the concentration of calibrator and increasing the dilution of the test specimen.

The primary dilutions provide enough volume for duplicate well ELISA testing.

Allow the kit components to equilibrate to room temperature (20-25°C).

1. Remove and assemble the required number of microwell strips from the Rat Kim-1 antibody coated plate, R-RENA-E-002, to perform the test. Two 8-well strips are required for negative control and calibrator testing in duplicate. Return the unused strips and desiccant to the foil pouch and reseal.
2. Wash strips 3X in Wash Solution, leave the wells full on the third wash, and allow to soak for 5-30 minutes.
3. Aspirate or shake-out the Wash Solution from the strips.
4. Add 50 µL/well Rat KIM-1 MAb Solution, Cat. No. RKMS-006, **blue dye**, to all wells of the test strips.
5. Add 50 µL/well KIM-1 Negative Control, Cat. No. HKNC-001, the diluted calibrator series and the diluted specimens, prepared previously, to the designated wells of the test strips as indicated in Figure 1. Tap gently to mix.
   Note: Dye color will change as controls, calibrators and specimens are added. Figure 1 illustrates using the recommended last (most dilute) seven calibrator dilutions which should cover the linear range. Any or all calibrators may be used as determined by the technician.

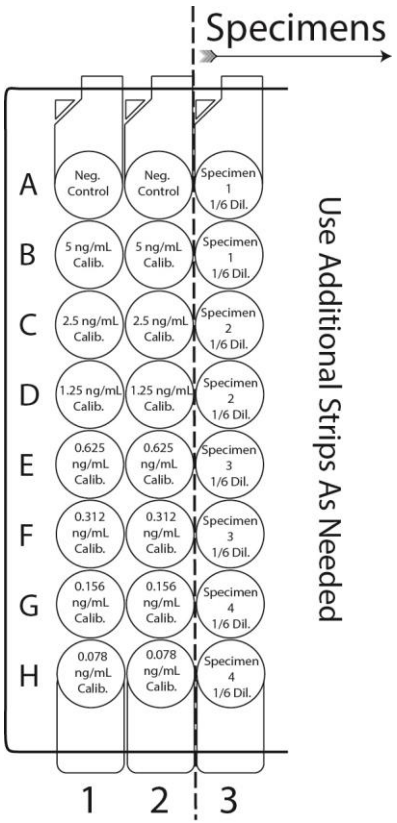

Figure 1 - Well configuration for duplicate well ELISA testing. Final concentrations in the wells are shown.

6. Incubate the strips at 37°C for 30 minutes in a waterbath or incubator - high humidity is required to avoid evaporation.
7. Aspirate or shake-out the contents of the strips and wash 5X with Wash Solution. Wash cycle is carried out as follows: aspirate or shake-out the contents of the wells and dispense 300-350 µL/well of Wash Solution, allow to soak for 10–30 seconds and aspirate or shake-out the contents. Repeat the wash cycle as indicated.
8. Add 100µL/well Peroxidase Conjugate Solution, Cat. No. PCS-012, to all wells of the test strips.
9. Incubate the strips at 37°C for 30 minutes in a waterbath or incubator - high humidity is required to avoid evaporation.
10. Wash 5X as indicated in step 7.
11. Add 100 µL/well ABTS Substrate Solution, Cat. No. KABTS-012, to all wells of the test strips.
12. Incubate the strips at room temperature (20-25°C) for 20 minutes.
13. Add 100 µL/well Stop Solution, Cat. No. KSS-012, to all wells of the test strips. Tap gently to mix.
14. Read optical density (O.D.) at 405 nm with a dual wavelength reference filter set at 490 nm. Blank the reader on the Negative Control well(s) or subtract the mean Negative Control O.D. from all O.D. readings.

Quality Control

A full calibration curve should be performed with each assay.

The optical density $O.D._{405/490\ nm}$ of the calibrator when diluted to 1.25 ng/mL should be ≥ 0.600.

The optical density readings of duplicate wells should be within 15%.

The ABTS Substrate Solution should be colorless or very faint green. Development of a green color may indicate contamination or instability.

Verify the linearity of the calibration curve generated in the Data Reduction section below. Significant variation in linearity may indicate pipet/calibrator concentration error. If this occurs, repeat the assay with freshly prepared calibrator dilutions.

Data Reduction

Most EIA plate readers are equipped with data reduction capability that can be used to generate the standard curve and provide linear-regression forecast/prediction of Kim-1 concentration. Plot the log of the mean O.D. reading for each calibrator concentration tested along the y-axis and the log of the concentrations in ng/mL along the x-axis.

Alternatively, you may email customer service at sales@bioassayworks.com to receive a spreadsheet to accurately plot specimen concentration from your laboratory data.

Typical Calibration Curve

Well #	Description	Mean O.D.	Conc. (ng/mL)
A1, A2	Neg. Control	0.001	0.0
B1, B2	Pos. Calib. #2	1.745	5.0
C1, C2	Pos. Calib. #3	1.326	2.5
D1, D2	Pos. Calib. #4	0.850	1.25
E1, E2	Pos. Calib. #5	0.504	0.625
F1, F2	Pos. Calib. #6	0.281	0.312
G1, G2	Pos. Calib. #7	0.158	0.156
H1, H2	Pos. Calib. #8	0.084	0.078

The shown calibration curve is for example only. Do NOT use the above values for data reduction.

References

1 - Vaidya et al., NATURE BIOTECHNOLOGY VOLUME 28 NUMBER 5 MAY 2010, "Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies."

2 – Vaidya et al., KIDNEY INTERNATIONAL VOLUME 76 (1) 8-10, 2009. "A rapid urine test for early detection of kidney injury."

Warranty

These products are warranted to perform as described in their labeling and in BioAssay Works®, LLC literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY, AND BIOASSAY WORKS®, LLC DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. BioAssay Works'®, LLC sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BioAssay Works®, LLC, to repair or replace the products. In no event shall BioAssay Works®, LLC be liable for any proximate, incidental or consequential damages in connection with the products.

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