Rat Kim-1 ELISA

Cat. No.: RRBA001R

1. BACKGROUND

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 by many organizations and agencies, including FDA, as an excellent tool in pre-clinical studies to monitor acute kidney tubular toxicity, by identifying adverse reactive drugs and therapeutic agents in drug development.

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 been shown time and again to outperform traditional biomarkers of kidney injury in preclinical biomarker studies.1 Rats injected with increasing doses of gentamicin, cadmium, mercury or chromium release into the urine proportionately increasing levels of Kim-1 antigen. The detection of Kim-1 can occur in as little as six hours post injection of an agent known to cause kidney injury.

2. REAGENTS PROVIDED TO PERFORM 80-88 TESTS FOR EACH PLATE

1. One (1) Rat Kim-1 antibody-coated plate
2. One (1) mL of Rat Kim-1 Calibrator/Control rehydration buffer
3. 100 µL (after formulation) Rat Kim-1 Positive Antigen-Control Urine, Note: see "Methods" section of label inside of kit- box lid for formulation instructions.
4. 100 µL Rat Kim-1 Negative Control Urine
5. Lyophilized Rat Kim-1 20 ng/mL Calibrator; Note: see "Methods" section of label inside of kit-box lid for rehydration instructions.
6. 10 mL Rat Peroxidase-Anti-Kim-1 Conjugate Solution, ready-to-use
7. 10 mL Sample Dilution Buffer, ready-to-use
8. 5 mL Rat KIM-1 Plate Stabilizer
9. 10 mL ABTS Substrate Solution, ready-to-use
10. 10 mL Stop Solution, ready-to-use
11. 25 mL 20X Wash Solution (dilute 1:20 with laboratory high-purity-grade water)

NOTE: Store all provided kit reagents at 2-8°C.

3. EQUIPMENT AND MATERIALS REQUIRED

a) High precision pipette (i.e., 1-20 microliter pipette)
b) 0.2 mL and 1.0 mL pipettes
c) 8- or 12-channel pipette (or transfer pipette)
d) 2 graduated cylinders (50 mL and 500 mL)
e) 1 mL or 5 mL borosilicate glass test tubes or plastic tubes; DO NOT use polypropylene
f) Uncoated low-binding 96-well plates (e.g, Nunc catalog # 269620)
g) Laboratory grade (distilled or R.O. deionized) water
h) A 96-well plate-reading spectrophotometer with 405 nm filter and 490 nm differential filter
i) Plate washing apparatus

4. PRECAUTIONS

a) Handle all reagents and samples as biohazardous material.
b) Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
c) Wash solution, control urine, sera, test plates, test samples and all other assay reagents should be properly decontaminated with bleach or other decontamination agent, or autoclaved before disposal.
d) Take special care not to contaminate any of the test reagents with urine or bacterial agents.
e) Use aseptic technique when handling samples.
f) Best results are achieved by following the protocol described below, using good, safe laboratory techniques.
g) Do not use this kit after the expiration date.

NEVER PIPETTE BY MOUTH

5. SAMPLE COLLECTION

For routine urine Kim-1 antigen assessment, it is recommended that urine samples be frozen at time of collection if not being used within four days. Proper sample collection procedures and storage (4°C for up to four days or -20°C for longer periods) are needed for reliable test results.

6. ASSAY PROCEDURE

NOTE: ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE BEFORE TESTING!

Preparation of the calibration curve

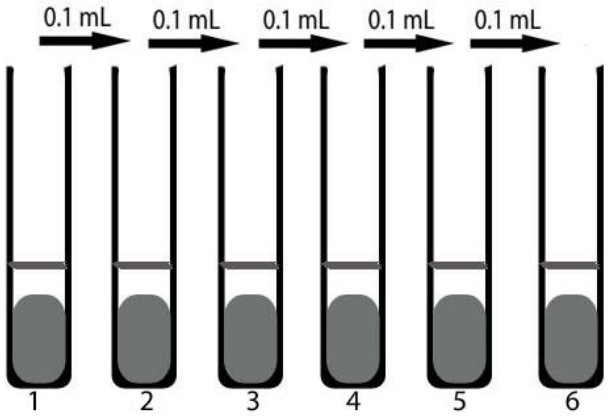
Using six (6) clean test tubes, label the

- **first tube "10 ng/mL**,"
- **second tube "5 ng/mL**,"
- **third tube "2.5 ng/mL**,"
- **fourth tube "1.25 ng/mL**,"
- **fifth tube "0.625 ng/mL,"**
- **sixth tube 0.313 "ng/mL**."

1) Add **100 µL** of the **Sample Dilution Buffer** to each tube. Using accurate pipetting technique and the below diagram.

2) Add **100 µL** of the **20 ng/mL calibrator** to the **first tube (10 ng/mL)** and mix using a vortexer.

3) Remove **100 µL** from the first tube, add to the **second tube (5 ng/mL),** and vortex to mix. Complete the serial dilution as shown in the diagram below.



7. SET-UP PLATE FOR THE SAMPLE- DILUTION PROCEDURE

1. Dilute urine samples 1:3 using Dilution Buffer in a **clean, uncoated** 96-well micro-titer plate (urine dilution plate, user-supplied). The test can be performed using single wells for the controls and calibrators; however, a more accurate method is to run the controls and calibrators in duplicate, and use the average of the duplicates to create the standard curve.
2. Set up samples, calibrators and controls such that wells **AI** and **A**2 contain the **Negative Control Urine** and wells **B1** and **B2** contain the **Positive Control Urine**.

NOTE: calibrator position for the Kim-1 antibody-coated plate:

- wells **C1** and **C2** contain the **10 ng Calibrator**,
- wells **D1** and **D2** contain the **5 ng Calibrator**,
- Wells **E1** and **E2** contain the **2.5 ng Calibrator**,
- wells **F1** and **F2** contain the **1.25 ng Calibrator,**
- wells **G1** and **G2** contain the **0.625 ng Calibrator,**
- wells **H1** and **H2** contain the **0.313 ng Calibrator.**

The remaining wells are to be used for sample testing.

8. PREPARATION OF THE URINE-DILUTION PLATE

1. Add **50 µL Sample Dilution Buffer** to each well of an **uncoated** 96-well microtiter plate,
2. Add **25 µL** of **Negative Control Urine** to wells **AI** and **A2,**
3. Add **25 µL** of **Positive Control Urine** to wells **B1** and **B2**,
4. Add 25 µl of the **10-nanogram Calibrator** to wells **C1** and **C2**,
5. Add 25 µl of the **5 nanogram Calibrator** wells **D1** and **D2**,
6. Add 25 µl of the **2.5 nanogram Calibrator** to wells **E1** and **E2**,
7. Add 25 µl of the **1.25 nanograms Calibrator** to wells **F1** and **F2**,
8. Add 25 µl of the **0.625 nanogram Calibrator** to wells **G1** and **G2**,
9. Add 25 µl of the **0.313 nanogram Calibrator** wells **H1** and **H2,**
10. Add **25 µL** of each urine sample to remaining wells, starting with well **A3** and ending with well **H12** (moving left to right, row by row of wells).
11. **Let samples equilibrate for 1 minute** and repeatedly tap plate to mix samples or mix on a rotary shaker.
12. **Rapidly Transfer 50 µl of each diluted sample** to a Kim-1 blocked/stabilized antibody-coated ELISA plate: see ELISA procedure below.

NOTE: Diluted urine samples should be tested within 8 hours of preparation.

9. PREPARATION OF 1X WASH SOLUTION (1:20)

Dilute **25 mL** concentrated **Wash Solution** in **475 mL** laboratory grade (distilled or R.O. deionized) water. **Mix well.**

Approximately 500 mL Wash Solution is needed for each 96-well ELISA plate when using an automated plate washer. If using a manual washer such as a Nunc hand-held washer, about 400 mL of 1X wash solution is needed.

10. SUBSTRATE SOLUTION

The Substrate Solution is ready-to-use. (Each plate will require approximately 10 mL Substrate Solution.)
NOTE: Allow Substrate Solution to equilibrate to room temperature before use.

11. STOP SOLUTION

The Stop Solution is ready-to-use. Swirl bottle to mix before use.

NOTE: Allow Stop Solution to equilibrate to room temperature before use.

12. ELISA TEST PROCEDURE

PREPARING THE TEST PLATE

1. Remove a Kim-1 antigen-coated test plate from foil pouch and label according to dilution plate identification.

Please Note: Discard pipette tips after each row of samples is transferred. Transfer of samples to the ELISA plate should be done as quickly as possible.

2. Add **50 µL Kim-1 Plate Blocker/Stabilizer** to each well of the test plate.
3. Using an 8- or 12-channel pipette, transfer **50 µL/well** of each of the Controls, Calibrators and diluted urine samples from the **Urine Dilution Plate** and mix by pipetting up and down.
4. Tap the plate gently to mix. Place plate in a zip lock bag with a moisture pad.
5. Incubate the plate for 120 minutes (2 hours) at room temperature.

Proceed to Wash-Step

WASH PROCEDURE

NOTE: The wash procedure is a very critical step in any ELISA. Please follow the below steps as directed.

6. Using an 8- or 12-channel hand-held washer/vacuum manifold (Nunc Immuno- wash Catalog number 470175 or comparable automatic washing device), vacuum out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.
7. Using an 8- or 12-channel hand-held washer/vacuum manifold or automated washer, fill each well with approximately **300 µL Wash Solution.**
8. Allow Wash Solution to soak in wells for ten seconds; then discard contents into an appropriate waste container containing bleach or other decontamination agent.
9. Tap inverted plate to ensure that all residual liquid is removed.

Repeat wash procedure three more times.

ADDITION OF ANTI-Kim-1 PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION

NOTE: Allow Conjugate, Substrate and Stop Solution to equilibrate to room temperature before use**.**

10. Using an 8- or 12-channel pipette (or transplating device), dispense **100 µL of ready-to-use Anti-Kim-1 Conjugate** into each assay well. Tap plate to mix well contents. Place plate in a zip lock bag with a moisture pad.
11. Incubate for 60 minutes at room temperature.
12. Wash as in WASH PROCEDURE **steps 6, 7, 8** and **9** above.

13. Using an 8 or 12-channel pipette (or transplating device), dispense **100 µl Substrate Solution** into each test well. Discard pipette tips.
14. Incubate at room temperature for 20 minutes.
15. Using an 8- or 12-channel pipette (or transplating device), add **100 µL** ready-to-use **Stop Solution** to each test well. Tap plate to mix well contents.
16. Allow bubbles to dissipate before reading plate.

13. MANUAL PROCESSING OF DATA

a) Read the plate using an ELISA plate reader set at 405 nm with a 490 nm differential filter. Be sure to blank the reader as directed by the reader instructions.
b) Calculate the average Positive Control Urine absorbance (Optical Density or O.D.) using the absorbance values of wells B1 and B2. Calculate the average Negative Control Urine absorbance using values obtained from wells Al and A2 if running duplicates. Record both averages.
c) Subtract the average Negative Control absorbance from the average Positive Control absorbance. The difference is the corrected Positive Control absorbance.

Examples:

1. Example Positive Control absorbance (wells B1 and B2) : 0.456, 0.450
   Average = (0.456 + 0.450) / 2 = 0.453
2. Example Negative Control absorbance (wells A1 and A2): 0.060, 0.066
   Average = (0.060 + 0.066) / 2 = 0.063
3. Corrected Positive Control absorbance: (0.453) - (0.063) = 0.390

14. RESULTS

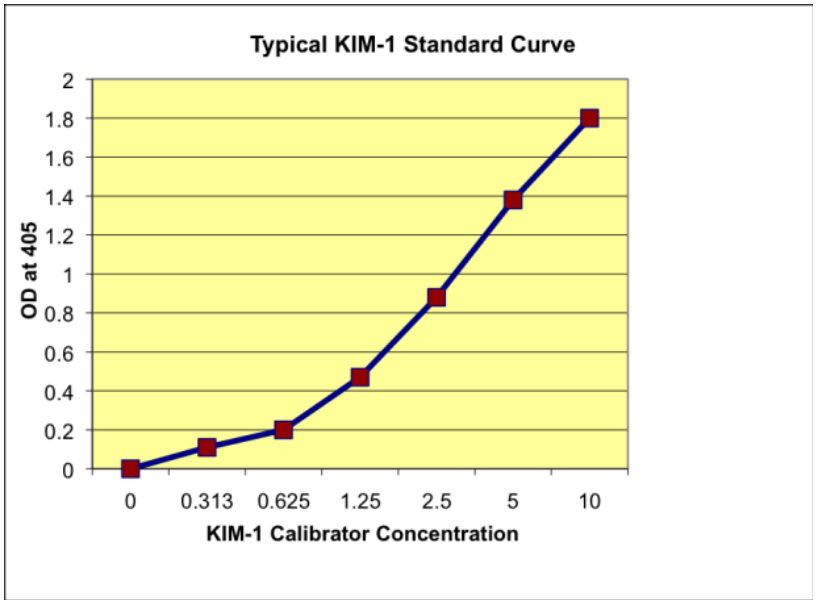
Assay Control Values

Valid Kim-1 ELISA results are obtained when the average optical density (O.D.) value of the Negative Control is less than 0.150 and the Positive Control concentration range is within the concentration range indicated on the label inside of kit-box lid.

If either of these values is out of range, the Kim-1 test results should be considered invalid, and the samples should be retested.

15. STANDARD CURVE GENERATION

In order to determine the concentration of Kim-1 antigen in nanograms per mL, a standard curve should be used for data reduction. Create the standard regression curve using appropriate software capable of generating a three-log dynamic range. Use values from the diluted calibrators to plot the curve. Use the Negative Control Urine for the zero (0) nanogram value. If any calibrator duplicates vary by more than 20% from each other, use only the single calibrator values that give the best curve fit.



16. 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”

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com