DATA SHEET

Human Kidney Injury Molecule 1 (KIM1) ELISA

For the quantitative determination of human KIM1 in urine

Cat. No. KT-562

For Research Use Only. Not for use in diagnostic procedures.

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PRODUCT INFORMATION

Human Kidney Injury Molecule (KIM1) ELISA

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INTENDED USE

The kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of human KIM1 in
urine. For research use only. Not for use in diagnostic procedures.

COMPONENTS

- Antibody-coated plate x 1
- Calibrator Diluent x 1 mL
- Positive control x 100 µL
- Negative control x 100 µL
- 20 ng/mL Calibrator x 1 (Lyoph.)
- Peroxidase-Anti-KIM1 Conjugate x 10 mL
- Sample Diluent x 10 mL
- Plate Stabilizer x 5 mL
- ABTS Substrate Solution x 10 mL
- Stop Solution x 10 mL
- 20X Wash Solution x 25 mL

MATERIALS REQUIRED BUT NOT SUPPLIED

1. High precision pipette (1-20 microliter pipette)
2. 0.2 mL and 1.0 mL pipettes
3. 8 or 12 channel pipette
4. 2 graduated cylinders (50 mL and 500 mL)
5. 1 mL or 5 mL borosilicate glass test tubes or plastic tubes;
Do not use polypropylene tubes
6. Uncoated low-binding 96-well plate
7. Distilled water
8. Plate reader with a 405 nm filter and a 490 nm differential filter
9. Plate washing apparatus

SAMPLE STORAGE

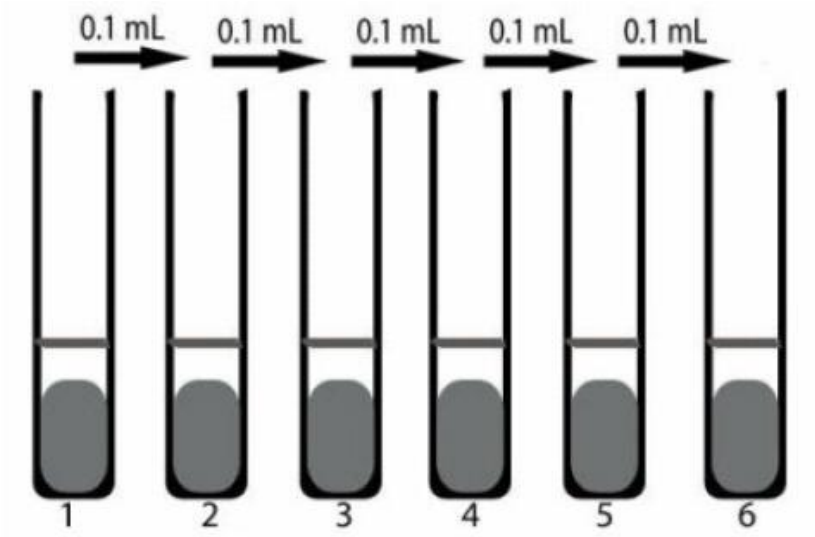
For routine urine KIM1 antigen assessment, it is recommended that urine samples be frozen at time of collection if not being used within four days. Proper sample collection procedures and storage (4°C for up
to four days or -20°C for longer periods) are needed for reliable test results.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.

Calibrator

Using six clean test tubes, label the tubes 10 ng/mL, 5, ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, and
0.313 ng/mL. Add 100 µL of Sample Diluent to each tube. Add 100 µL of the 200 ng/mL calibrator to the
first tube (10 ng/mL) and mix using a vortexer. Remove 100 µL from the first tube and add to the second tube (5 ng/mL), and vortex to mix. Complete the serial dilution as shown in the diagram below.



Uncoated Plate

Add 50 µL of Sample Diluent to each well of an uncoated 96-well microtiter plate. Add 25 µL of Negative
Control to wells A1 and A2. Add 25 µL of Positive Control to wells B1 and B2. Add 25 µL of 10 ng/mL calibrator to wells C1 and C2. Add 25 µL of 5 ng/mL calibrator to wells D1 and D2. Add 25 µL of 2.5 ng/mL calibrator to wells E1 and E2. Add 25 µL of 1.25 ng/mL calibrator to wells F1 and F2. Add 25 µL of 0.625 ng/mL calibrator to wells G1 and G2. Add 25 µL of 0.313 ng/mL calibrator to wells H1 and H2. Add 25 µL of each urine sample to the remaining wells. Start with well A3 and end with well H12 (moving
left to right, row by row of wells). Let samples equilibrate for 1 minute and repeatedly tap plate to mix samples or mix on a rotary shaker. Rapidly transfer 50 µL of each diluted sample to a KIM1 antibody coated plate: See ELISA Procedure.

Note:

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

Wash Solution

Dilute 25 mL concentrated Wash Solution in 475 mL distilled or deionized water. Mix well. Approximately
500 mL of 1x Wash Solution is needed for each 96-well ELISA plate when using an automated plate washer. If using a manual washer such about 400 mL of 1x Wash Solution is needed.

ASSAY PROCEDURE

1. Allow all reagents to equilibrate to room temperature before use.
2. Remove a KIM1 antigen-coated plate from foil pouch and label according to dilution plate identification.
3. Add 50 µL KIM1 Plate Stabilizer to each well of the test.
4. Using an 8 or 12 channel pipette, transfer 50 µL/well of each of the controls, calibrators, and diluted urine samples from the uncoated microtiter plate and mix by pipetting up and down.
5. Tap plate gently to mix. Place plate in a zip lock bag with a moisture pad.
6. Incubate for 120 minutes (2 hours) at room temperature.
7. Using an 8 or 12 channel hand-held washer/vacuum manifold, vacuum our liquid from each well into an appropriate vessel containing bleach or other decontamination agent.
8. Using an 8 or 12 channel hand-held washer/vacuum or automated washer, fill each well with approximately 300 µL Wash Solution.
9. Allow Wash Solution to soak in wells for ten seconds; then discard contents into an appropriate waste container containing bleach or other decontamination agent.
10. Tap inverted plate to ensure that all residual liquid is removed.
11. Repeat wash procedure three more times.
12. Using an 8 or 12 channel pipette dispense 100 µL of Anti-KIM1 conjugate into each assay well. tap plate to mix contents. Place plate in a zip lock bag with a moisture pad.

13. Incubate for 60 minutes at room temperature.
14. Wash as in steps 7-11 above.
15. Using an 8 or 12 channel pipette dispense 100 µL Substrate Solution into each well.
16. Incubate at room temperature for 20 minutes.
17. Using an 8 or 12 channel pipette dispense 100 µL Stop Solution into each well. Tap plate to mix contents.
18. Allow bubbles to dissipate before reading the plate.

CALCULATION OF RESULTS

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. Calculate the average Positive Control Urine absorbance OD using the absorbance values of wells B1 and B2. Calculate the average Negative Control Urine absorbance using values obtained from wells A1 and A2 if running duplicates. Subtract the
average Negative Control absorbance from the average Positive Control absorbance. The difference is
the corrected Positive Control absorbance.
Valid KIM1 ELISA results are obtained when the average optical density (OD) 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 the kit-box lid. If either of these values is out of range, the KIM1 test results should be considered invalid, and the samples should be retested.
In order to determine the concentration of KIM1 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 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.

IMPORTANT NOTES

1. Handle all reagents and samples as biohazardous material.
2. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
3. 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.
4. Take special care not to contaminate any of the test reagents with urine or bacterial agents.
5. Use aseptic technique when handling samples.
6. Best results are achieved by following the protocol described below, using good, and safe laboratory
techniques.
7. Do not use this kit after the expiration date.
8. Never pipette by mouth.

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