SMC™ Human KIM-1 Immunoassay Kit Instructions

Microparticle Assay

Catalog # 03-0118-00

Immunoassay kit for the quantitative determination of **Human Kidney Injury Molecule 1 (KIM-1)** in human serum, plasma and urine

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

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INTRODUCTION

The SMC™ Human Kidney Injury Molecule 1 (KIM-1) Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure KIM-1 in human serum, plasma and urine samples. A capture antibody specific for human KIM-1 has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the KIM-1 present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to KIM-1 that has been captured onto the beads. Following a stringent wash step to remove unbound detection, the beads are transferred to a clean plate. After a final aspirate, elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the bead surface releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the Erenna® System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of KIM-1 present in the sample when captured. The amount of KIM-1 in unknown samples is interpolated from a standard curve.

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REAGENTS

The SMC™ Human Kidney Injury Molecule 1 (KIM-1) Immunoassay Kit includes all reagents listed in Reagents Provided. Additional reagents and supplies may be required to run this immunoassay, as listed in the section titled General Supplies Required But Not Provided. All reagents supplied are for Research Use Only.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Assay Buffer	With cold pack	2 - 8°C	02-0807-00	1 x 50 mL
2	Human KIM-1 Coated Antibody	With cold pack	2 - 8°C	02-0801-00	1 x 50 µL
3	Assay Plate	With cold pack	2 - 8°C	02-0799-00	1 each
4	Blocking Buffer	With cold pack	2 - 8°C	02-0836-00	1 x 30 mL
5	Human KIM-1 Detection Antibody	With cold pack	2 - 8°C	02-0803-00	1 x 20 µL
6	Human KIM-1 Standard	On dry ice	≤ -70°C	02-0800-00	1 x 20 µL
7	10X Wash Buffer (Note: Contains 0.5% Proclin)	With cold pack	2 - 8°C	02-0001-06	2 x 50 mL
8	Buffer C	With cold pack	2 - 8°C	02-0230-01	1 x 3 mL
9	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	2 x 5 mL

Storage Instructions

- The SMC™ Human Kidney Injury Molecule 1 (KIM-1) Immunoassay Kit should be stored at 2 - 8°C. The Standard analyte should be stored at ≤ -70°C.
- Discard standards after one use.
- Proper kit performance can only be guaranteed if the materials are stored properly.

REAGENTS (continued)

General Supplies Required But Not Provided

Reagents

1. Erenna® 10X System/Wash Buffer w/ Proclin (1 L bottle) (EMD Millipore PN 02-0111-03)
2. Erenna® 10X Wash Buffer (1 L bottle) (EMD Millipore PN 02-0111-00) if using an automated plate washer
3. Elution Buffer (EMD Millipore PN 02-0002-04) for maintenance
4. De-ionized or distilled water

Washing Options

Automated

a. Bio-Tek ELx405™ Microplate Washer (EMD Millipore PN 95-0004-05) *or*
b. Tecan HydroFlex™ microplate washer (EMD Millipore PN 95-0005-02)

Manual

a. Sphere Mag Plate SBS Footprint (EMD Millipore PN 90-0003-02) *or*
b. DynaMag™-96 Side Skirted Magnet (Thermo Fisher PN 12027)

Instrumentation / Materials

1. Jitterbug™ Microplate incubator / shaker (EMD Millipore PN 70-0009-00 or equivalent)
2. ALPS™ 50V microplate heat sealer (Thermo Fisher PN AB1443A or equivalent)
3. Centrifuge with plate rotor capable of reaching a speed of 1,100 xg
4. 12-channel pipettes capable of transferring 20 µL - 250 µL
5. 8- or 12-channel pipette capable of transferring 15 µL
6. Rotisserie rotator
7. Microcentrifuge
8. MultiScreen$_{HTS}$ BV 96-Well Filter Plate (EMD Millipore PN MSBVN1210 or equivalent)
9. 96-well V-bottom polypropylene plate, 500 µL (Axygen PN P-96-450V-C)
10. 384-well round bottom polypropylene plate, 120 µL (Thermo Fisher PN 264573)
11. 0.2 µm syringe filter (EMD Millipore PN SLGPR33RS or equivalent)
12. Universal plate cover (Thermo Fisher PN 253623 or equivalent)
13. Sealing tape (Thermo Fisher PN 236366 or equivalent)
14. Heat sealing plate foil (EMD Millipore PN 02-01-0216-00 or equivalent)
15. 12-channel reagent resevoirs for preparing standards
16. 5 mL syringe
17. Microcentrifuge tubes
18. Container capable of holding 300 mL
19. 500 mL graduated cylinder

(Please contact your technical services representative for additional information or assistance selecting required but not provided supplies.)

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set-up.

- Wipe down bench and pipettes with 70% isopropanol before use. It is important to allow all reagents to warm to room temperature (20 - 25°C).
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring standard.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation. It is recommended that the standards are prepared as the last step prior to plate setup.
- The detection antibody is light sensitive and must be protected from light at all times.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the wash buffer provided.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- The plates should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate with the pierceable foil seal and store the plate at 2-8°C for up to 48 hrs. Bring to room temperature then centrifuge the plate at 1,100 x g for 5 minutes prior to reading on the Erenna.
- The plate shaker should be set at a speed to provide maximum orbital mixing without splashing liquid on the sealer or outside the wells. For the recommended plate shaker, this would be a setting of 3 - 5.
- For optimal instrument performance, complete a cycle routine (10,000 µL at 1,000 µL/min) followed by a bubble test, and an instrument calibration prior to reading the plate.
- If a clean routine is required, run using three wells of elution buffer (EMD Millipore PN 02-0002-04), one well of 10% bleach and five wells of elution buffer (EMD Millipore PN 02-0002-04). (Note: This elution buffer is not provided and should be ordered separately.)

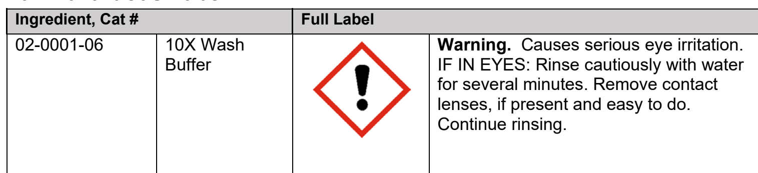
SAMPLE INFORMATION

- The SMC™ Human KIM-1 Immunoassay validation data have been compiled using serum, plasma and urine.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the SMC™ Human Kidney Injury Molecule 1 (KIM-1) Immunoassay.

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.
- Proclin-containing solutions and their containers must be disposed of in a safe way and in accordance with local, regional and national regulations.
- The chemical, physical and toxicological properties Proclin 950 at 5% have not been thoroughly investigated. At this concentration, this biocidal preservative is irritating to eyes and skin, and may be detrimental if enough is ingested (quantities above those found in the kit). ProClin 950 is a potential sensitizer by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals. The potential for these adverse health effects is unknown for the highly diluted, small volume of ProClin in this kit, but unlikely if handled appropriately with the requisite good laboratory practices and universal precautions. For full concentration information, please refer to the SDS.

Full Hazardous Label:

Ingredient, Cat #		Full Label	
02-0001-06	10X Wash Buffer		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

ASSAY PREPARATION

Microplate Coating

1. Prepare carbonate-bicarbonate buffer (0.05 M, pH 9.6). For example, dissolve 1 Sigma C3041 capsule into 100 mL deionized water.
2. Warm the Human KIM-1 Coating Antibody to room temperature prior to use.
3. Dilute the Coating Antibody 1: 200 in Bicarbonate Buffer (for example, transfer 35 µL Coating Antibody into 6.965 mL of Bicarbonate Buffer).
4. Remove the microplate from the package.
5. Pipette 50 µL of diluted coating antibody into each well of the assay plate. Ensure that the solution has evenly coated the bottom of each well.
6. Seal the plate and incubate without shaking overnight at 4°C.
    a. NOTE: For consistent results, use this microplate and coating condition. Other configurations have not been tested.

Reagent Preparation

1. Warm the following reagents to room temperature prior to use: Coated Assay Plate, Blocking Buffer, Assay Buffer, Elution Buffer B, Buffer C, Detection Antibody and 10X Wash Buffer.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour 100 mL of the 10X Wash Buffer into a container capable of holding at least 1 L.
    b. Add 900 mL of deionized water.
    c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar.
    NOTE: 10X Wash Buffer does not contain preservative. After dilution, the 1X wash buffer may be filter sterilized (Stericup® filter, EMD Millipore PN SCGPU11RE) for storage of up to 1 month at 2 - 8°C. If not filter sterilized, all remaining 1X Wash Buffer should be discarded upon experiment completion.
4. Wash the plate 3 times with 250 µL 1X Wash Buffer. This can be done manually or with an automated plate washer. Ensure that the well is free of residual volume.
5. Block the plate with 200 µL/well of Blocking Buffer. Incubate with shaking for 1 hour at 25°C on Boekel Scientific, The Jitterbug™ setting 4.

ASSAY PREPARATION (continued)

Sample Preparation

1. Prepare samples by one of the following methods:
    a. Plasma and Serum samples should be diluted 1:2 in Assay Buffer prior to measurement.
    b. Urine samples should be diluted 1:10 in Assay Buffer prior to measurement.
    c. If using a filter plate with prefilter (EMD Millipore PN MSBVN1210 or equivalent): Stack the filter plate on top of a 96-well receptacle plate. Place ≤ 250 μL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x g.
    d. If using a microcentrifuge: Centrifuge samples at >13,000 x g for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

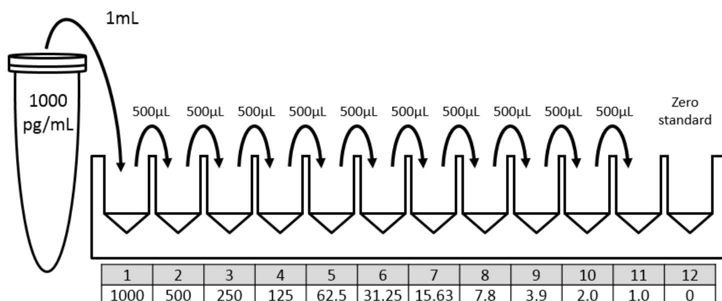
Initial Standard Stock Preparation

1. Quick spin the KIM-1 Standard vial in a microcentrifuge and pipette mix prior to preparing standards. Use care when opening the stock standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the KIM-1 Standard in the vial.
3. To make your Analyte Working Stock, perform the necessary serial dilutions, in Assay Buffer, to achieve the final working concentration of 1,000 pg/mL in a 1 mL final volume. Ensure that all pipetting steps transfer ≥10 μL of liquid to achieve the best precision.
4. Discard standard after one use.

HUMAN KIM-1 ASSAY PROCEDURE

Standard Curve

Prepare the standard curve dilutions in a 12-channel reservoir. Perform 1:2 serial dilutions of the Analyte Working Stock for standards 2 through 11 to achieve a curve from 1,000 pg/mL to 1.0 pg/mL. Run the standards in triplicate.



1	2	3	4	5	6	7	8	9	10	11	12
1000	500	250	125	62.5	31.25	15.63	7.8	3.9	2.0	1.0	0

1. Add 500 µL Standard Diluent to wells 2 through 12 of a 12-channel reservoir.
2. Add 1,000 µL of the 1,000 pg/mL Analyte Working Stock from standard preparation into well 1.
3. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2, stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

Target Capture

1. Wash the plate 3 times with 250 µL 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.
2. Pipette 50 µL per well of Standards or diluted Samples to Assay Plate.
3. Cover Plate 1 with a plate sealing film.
4. Incubate for 2 hours at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. Approximately 10 minutes prior to the end of Target Capture incubation, prepare the Detection Antibody. Add 15 µL of Detection Antibody to 60 µL of Assay Buffer to make a 1:5 dilution. Then add 70 µL of diluted antibody to 6930 µL of Assay Buffer.
6. When target capture incubation is complete, carefully remove temporary plate cover to avoid splashing.

HUMAN KIM-1 ASSAY PROCEDURE (continued)

Detection

1. Wash the plate 6 times with 250 µL 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.
2. Add 50 µL per well of Detection Antibody.
3. Cover Plate 1 with plate sealing film.
4. Incubate for 1 hour at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. Carefully remove plate sealing film to avoid splashing.

Elution and Plate Transfer

1. Wash the plate 6 times with 250 µL 1X Wash Buffer. This can be performed manually or with an automated plate washer. Ensure that the well is free of residual volume.Immediately remove Plate 2 from the magnet.
2. Add 50 µL Elution Buffer B per well.
3. Cover Assay Plate with a plate sealing film.
4. Incubate plate for 10 minutes at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. While the Assay Plate is incubating, add 10 µL per well of Buffer C to Plate 3 (384-well polypropylene plate (Thermo Fisher PN 264573 or equivalent)) using a 12-channel manual P20.
6. Set manual 8-channel pipette to 30 µL and transfer eluate to Read Plate by columns.
7. Cover Read Plate with a universal plate cover and spin plate for 5 minutes at RT, approximately 1,100 x g.
8. Cover Read Plate with heat sealing foil (EMD Millipore PN 02-01-0216-00 or equivalent), according to manufacturer's instructions for the heat sealer.

Run on Erenna® Immunoassay System

1. Load completed assay Read Plate onto the Erenna® Immunoassay System.

APPENDIX A: SMC™ Quick Assay Guide

1. Prepare Microplate coating. Add 50 µL of **Diluted Coating Antibody** to each well.
2. Coat sealed plate and incubate for overnight at 4°C on microplate incubator/shaker. **Do Not Shake**

Overnight 4°C

3. Wash **Assay Plate** 3 times with 250 µL **Wash Buffer**.
4. Block plate with 200 µL/well of **Blocking Buffer** for 1 hour at 25°C on microplate incubator/shaker.
5. Prepare all reagents, standard curver and samples as directed.

1 hour 25°C

6. Wash **Assay Plate** 3 times with 250 µL **Wash Buffer**
7. Add 50 µL of Standard/Diluted Samples to **Assay Plate**.
8. Cover and incubate for 2 hours at 25°C on microplate incubator/shaker.

2 hours 25°C

9. Wash **Assay Plate** 6 times with 250 µL **Wash Buffer**.
10. Add 50 µL of **Detection Reagent** per well.
11. Cover and incubate for 1 hour at 25°C

1 hour 25°C

12. Wash **Assay Plate** 6 times with 250 µL **Wash Buffer**.
13. Add 50 µL of Elution Buffer B per well.
14. Cover and incubate for 10 minutes at 25°C

10 minutes 25°C

15. Add 10 µL Buffer C per well to Read Plate
16. Transfer contents of Assay Plate to Read Plate.
17. Cover assay Read Plate with pierceable plate seal cover.
18. Cover and centrifuge for 5 minutes at 1,100 x g.

LOAD ON ERENNA® SYSTEM

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipeting with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash and system buffers) are not contaminated.
		Change tips for each dilution of the standard curve.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Instrument needs cleaning	See Technical Guidelines for appropriate Erenna® cleaning protocol.
	Plate was over-incubated	Confirm correct incubation times were followed.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be centrifuged or filtered according to the PI and lab SOPs. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing (~650 - 1000 RPM).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. Should splashing occur on the plate sealer pulse spin plate to remove excess material prior to removing the seal. A new plate seal should be used every time the plate is sealed.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Sample variability is high (continued)	Cross-well contamination (continued)	Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
Published LLoQ was not achieved	Improper dilution/reconsititution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains < 2 µL following the post-capture and final aspiration protocols.
		Ensure time from thawing the standard to starting the capture incubation is ≤10 minutes.

ORDERING INFORMATION

To place an order or to obtain additional information about SMC™ products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds

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