Luminex® Performance Assay

Rat Kidney Toxicity Premixed Kit

Catalog Number FCST15

For the simultaneous quantitative determination of multiple rat kidney toxicity biomarkers in serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Drug toxicity commonly causes major organ damage to the kidneys. In a clinical setting, nephrotoxicity can potentially contribute to acute kidney injury and possibly chronic renal disease (1,2). During drug development, nephrotoxicity in animal models is a major factor in the failure of many prospective compounds. The customary method for monitoring drug-induced nephrotoxicity in preclinical toxicology studies has been to measure serum creatinine (SCr) and blood urea nitrogen (BUN) levels. However, these renal functional biomarkers lack the sensitivity and specificity to adequately detect nephrotoxicity before significant kidney damage has occurred (2-4). This kit assesses the levels of five key renal toxicity protein markers that are more sensitive than SCr and BUN (2,5,6).

This kit is an excellent tool for drug toxicology studies because it can simultaneously assess the levels of TIM-1, Cystatin C, FABP1, Lipocalin-2, and Osteopontin in a single serum, plasma, or urine sample. For ease of use, the microparticles and the biotinylated detection antibodies are premixed in respective vials.

Analyte	Microparticle Region	Performance Data Online (www.RnDSystems.com/pdf/...)
FABP1/L-FABP	31	LRK1565.pdf
Lipocalin-2/NGAL	12	LRK3508.pdf
TIM-1/KIM-1/HAVCR*	25	LRK3689.pdf
Cystatin C	35	LRK6154.pdf
Osteopontin	51	LRK6359.pdf

PRINCIPLE OF THE ASSAY

Luminex® Performance Assay multiplex kits are designed for use with the Luminex 100™, Luminex 200™, or Bio-Rad® Bio-Plex® dual laser, flow-based sorting and detection analyzers.

Analyte-specific antibodies are pre-coated onto color-coded microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated detection antibodies, is added to each well. A final wash removes unbound Streptavidin-PE. The microparticles are resuspended in buffer and read using the Luminex or Bio-Plex analyzer. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of analyte bound.

**This product is covered by one or more of the following US Patents: 7,300,652; 7,041,290; 6,664,385; and other US and foreign patents pending or issued.*

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. However, until these factors have been tested in the Luminex Peformance Assay, the possibility of interference cannot be excluded.
- Luminex Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a single complex sample. A single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit. Refer to the enclosed certificate of analysis for specific analytes included in this kit.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.
- For best results, adjust the vacuum strength on the plate washer to between 15 and 40 cm of mercury.

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Standard Cocktail	893835	2 vials of recombinant rat kidney toxicity biomarkers in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard for each assay.
Microparticle Cocktail; Rat Kidney Toxicity Premixed Kit	894566	1.2 mL of a concentrated microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Once diluted, any unused microparticle cocktail must be discarded.*
Biotin Antibody Cocktail; Rat Kidney Toxicity Premixed Kit	894565	1.2 mL of a concentrated biotin antibody cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	11 mL of a buffered protein base with preservatives. *Use to dilute microparticles.*	
Biotin Antibody Diluent 3	895955	5 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-48	895579	21 mL of a 2-fold concentrated solution in a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	
Streptavidin-PE	892525	0.07 mL of a 100-fold concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	640763	1 filter-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent.	
Plate Sealers	640445	6 adhesive foil strips.	
Standard Value Card	749124	1 card listing the Standard Cocktail reconstitution volume and working standard concentrations for this kit lot.	

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Luminex 100, Luminex 200, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Microplate vacuum manifold (Millipore Multiscreen™ Vacuum Manifold Catalog # MAVM096 or equivalent).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Allow blood samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin and citrate plasma have not been validated for use in this assay.*

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

If samples contain particulate matter, centrifuge at 5000 x g for 2 minutes prior to use.

FABP1 and TIM-1 samples require a 4-fold dilution. A suggested 4-fold dilution is 60 µL of sample + 180 µL of Calibrator Diluent RD6-48 (diluted 1:2).* Mix thoroughly.

Cystatin C, Lipocalin-2, and Osteopontin samples require a 200-fold dilution. A suggested 200-fold dilution can be achieved by performing the 4-fold dilution shown above followed by adding 10 µL of diluted sample to 490 µL of Calibrator Diluent RD6-48 (diluted 1:2). Mix thoroughly.

*See Reagent Preparation section.

REAGENT PREPARATION

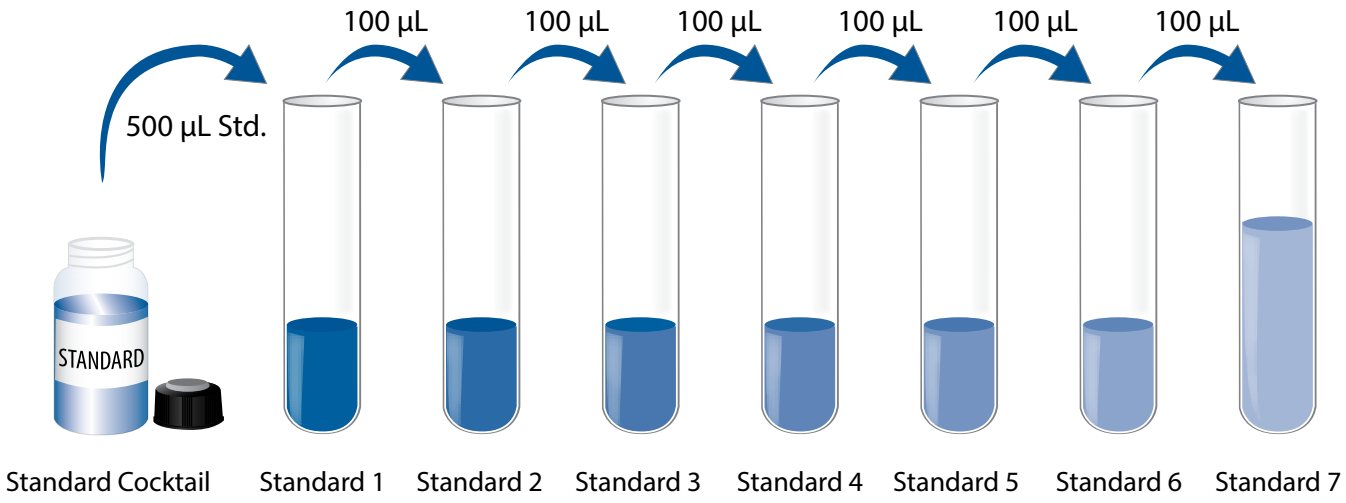
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD6-48 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD6-48 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-48 (diluted 1:2).

Standard - Reconstitute the Standard Cocktail with Calibrator Diluent RD6-48 (diluted 1:2). Refer to the Standard Value Card for the reconstitution volume. Allow the standards to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of the reconstituted Standard into the Standard 1 tube. Pipette 200 µL of Calibrator Diluent RD6-48 (diluted 1:2) into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD6-48 (diluted 1:2) serves as the blank. Refer to the Standard Value Card for the assigned value of Standard 1.
Use within four hours of preparation.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

Note: *Prepare diluted Biotin Antibody Cocktail and diluted Microparticle Cocktail at the same time.*

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Assay Diluent RD1W
96	1000 µL	+	9.5 mL
72	750 µL	+	7.125 mL
48	500 µL	+	4.75 mL
24	250 µL	+	2.375 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin Antibody Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin Antibody Cocktail in Biotin Antibody Diluent 3. Mix gently.

Number of Wells Used	Biotin Antibody Cocktail	+	Biotin Antibody Diluent 3
96	1000 µL	+	4.5 mL
72	750 µL	+	3.375 mL
48	500 µL	+	2.25 mL
24	250 µL	+	1.125 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the 100X Streptavidin-PE to a 1X concentration by adding 55 µL of Streptavidin-PE to 5.5 mL of Wash Buffer.

INSTRUMENT SETTINGS

Adjust the probe height setting on the Luminex analyzer to avoid puncturing the membrane. Refer to the instrument manual.

a) Assign the bead region for each analyte being measured (see page 1)

b) 50 events/bead

c) Minimum events: 0

d) Flow rate: 60 µL/minute (fast)

e) Sample size: 50 µL

f) Doublet Discriminator gates at approximately 7500 and 15,500

g) Collect Median Fluorescence Intensity (MFI)

Note: *For the Bio-Rad Bio-Plex analyzer, set the gates at 4300 and 10,000. The CAL2 setting for the Bio-Rad Bio-Plex analyzer should be set at the low RP1 target value.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Pre-wet the filter-bottomed microplate by filling each well with 100 µL of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accomodate a microplate.

   Note: *After each final wash cycle and subsequent reagent addition, blot the bottom of the plate with a paper towel to prevent wicking.*
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 100 µL of the microparticle mixture to each well of the filter-bottomed microplate provided.
4. Add 50 µL of Standard or sample* per well. Pipette assay within 15 minutes. Securely cover with a foil plate sealer. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Using a vacuum manifold device designed to accommodate a microplate, wash by removing the liquid, filling each well with Wash Buffer (100 µL) and removing the liquid again. All of the liquid must be removed through the filter at the bottom of the plate to avoid any loss of microparticles. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.
6. Add 50 µL of diluted Biotin Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 500 ± 50 rpm.
7. Repeat the wash as in step 5.
8. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm.
9. Repeat the wash as in step 5.
10. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 500 ± 50 rpm.
11. Read within 90 minutes using the Luminex or Bio-Rad analyzer.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This assay is calibrated against highly purified recombinant rat kidney toxicity biomarkers produced at R&D Systems.

REFERENCES

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.