ab193654 – Rat AKI Antibody Array - Membrane (7 Targets)

Instructions for Use

For the simultaneous detection of 7 Rat AKI protein biomarkers in serum, plasma and urine samples

This product is for research use only and is not intended for diagnostic use.

Version 3a Last Updated 26 November 2024

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1. BACKGROUND

Abcam's Rat AKI Antibody Array - Membrane (7 Targets) ab193654 can be used for the simultaneous detection of 7 rat AKI antibody biomarkers. Suitable for all liquid sample types.

Targets: Cystatin C, FABP1, TIM-1 (KIM-1), MCP-1 (CCL2), Lipocalin-2 (NGAL), TIMP-1, VEGF-A

Acute kidney injury is a common complication among ambulatory and hospitalized patients. It is a rapidly progressive illness that independently predicts excess morbidity and mortality. It is critical to early detect acute kidney injury and distinguish it from prerenal azotemia and chronic kidney disease at the time of patient presentation to rapidly manage associated illness. However, serum creatinine, a standard marker of kidney function, does not distinguish acute kidney injury from prerenal azotemia or chronic kidney disease. In addition, the initial measurement of serum creatinine cannot reflect the extent of injury because its accumulation always lags behind the insult.

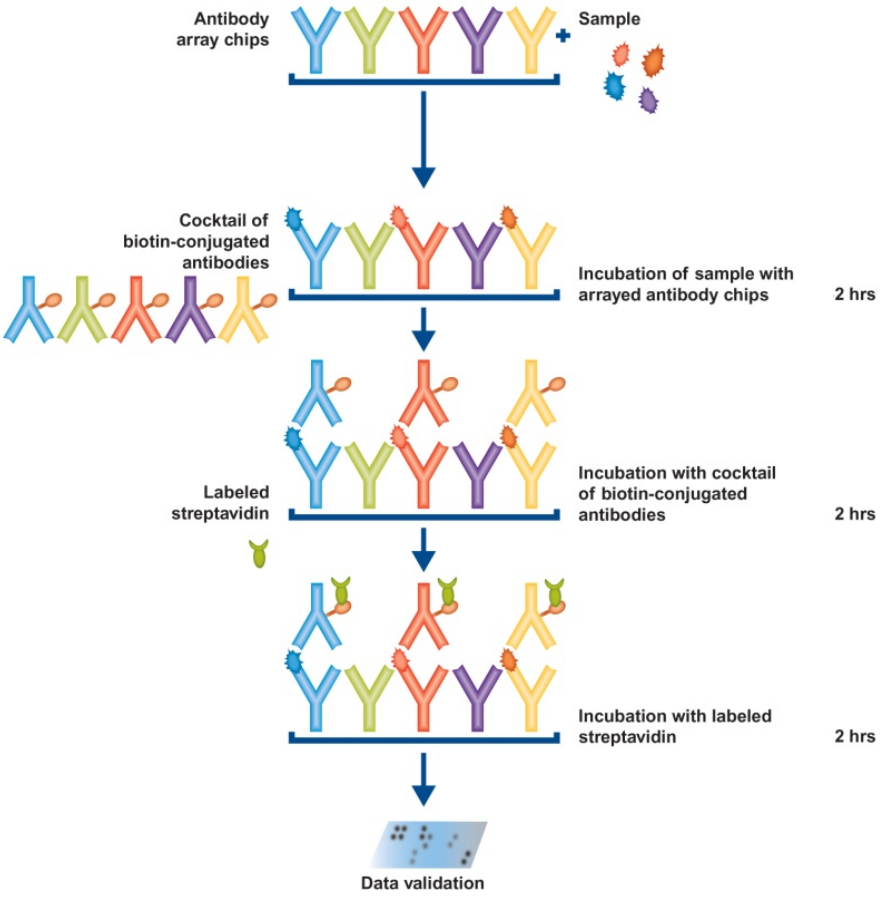
The kidney is the primary organ responsible for the excretion of medications and their biotransformation products from the body.

Rats are widely used for probing pharmacokinetic-pharmacodynamic (PK-PD) relationships for medications; in addition, rats have been demonstrated to be a useful model for evaluating mechanisms of kidney toxicity. In recent years, numerous molecules have been described and investigated as candidate biomarkers of kidney injury. The United States Food and Drug Administration (FDA) has taken an active role in developing a process for qualification of biomarkers that would potentially improve the drug development and regulatory review process. In the gentamicin-induced rat model of acute kidney injury, based on histopathology, necrosis, or apoptosis scoring, kidney injury molecule-1 (KIM-1) was the best biomarker of overall renal injury.

Traditionally, urine proteins or cytokines are detected by using ELISA. However, Abcam's Rat AKI Antibody Array - Membrane (7 Targets) can detect 7 protein biomarkers simultaneously with a

small amount of sample. It is a great tool in the acute kidney injury research areas and drug discovery area to hasten drug development.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC immediately upon receipt.

Once thawed, for short-term storage, store array membranes and 1X Blocking Buffer at ≤ -20°C, and all other component at 2-8°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. MATERIALS SUPPLIED

Item	Quantity		Storage Condition (Before Preparation)
	4X Membranes	8X Membranes	
Rat AKI Antibody Array Membranes (C1)	4X C1 Membranes	8X C1 Membranes	-20ºC
2000X Biotin-Conjugated Anti-Cytokines (C1)	2X C1 Vials	4X C1 Vials	-20ºC
1000X HRP-Conjugated Streptavidin	1 Vial	1 Vial	-20ºC
1X Blocking Buffer	25 mL	50 mL	-20ºC
20X Wash Buffer I	10 mL	20 mL	-20ºC
20X Wash Buffer II	10 mL	20 mL	-20ºC
Detection Buffer C	1.5 mL	2.5 mL	-20ºC
Detection Buffer D	1.5 mL	2.5 mL	-20ºC
8-Well Plastic Tray	1 Unit	1 Unit	-20ºC
Plastic sheets	1 Unit	1 Unit	-20ºC

The kit also includes plastic sheets, a booklet, an array template and a packing list.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Small plastic boxes or containers.
- Pipettors, pipet tips and other common lab consumables.
- Orbital shaker or oscillating rocker.
- Saran Wrap or similar plastic film.
- A chemiluminescent blot documentation system.
- X-ray Film and a suitable film processor.

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

Handling Array Membranes

- Array membranes are fragile when dry. Handle with care.
- Wet or dry, grasp membranes by the edges using forceps.
- Do not allow membranes to dry out during experiments.
- The printed side of each membrane is denoted by a dash mark (-) or array number in the upper left corner.

Incubation and Washes

- All washes and incubations in the standard protocol can be performed using the 8-well tray provided in the kit.
- Place the cover on 8-well trays with lid to avoid drying, particularly during extended incubation or wash steps.
- During each incubation, be sure to completely cover the membranes with sample or reagent.
- During incubation steps, avoid foaming and be sure to remove all bubbles from the membrane surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (≈0.5 to 1 cycles/second).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 2-8°C. Overnight sample incubations are the most effective at increasing antigen-specific spot intensities.
- If you perform overnight sample incubations, we recommend adding the optional “Large Volume Wash” described in Step 12.5 to minimize background signals.
- Overnight blocking and wash steps are useful for reducing background signal intensities. Wash steps may be repeated even with completed membranes to reduce background signals. Wash with Wash Buffer II, followed by repeating incubation with Streptavidin-HRP and chemiluminescent detection may greatly improve signal-to-noise ratios in your developed array images.

Chemiluminescence Detection

- We strongly recommend using multiple exposures to obtain optimum images. Begin by exposing the membranes for 40 seconds. Then re-expose the film accordingly.
- If the signals are too strong (or background is too high), reduce exposure time (e.g. 5-30 seconds).
- If the signals are weak, increase exposure time.
- Blot documentation systems that use CCD cameras to detect chemiluminescence are ideal for imaging Abcam array membranes. They can easily be programmed to take multiple exposures, and the dynamic range of these detectors tends to be 2-3 orders of magnitude greater than that of X-ray film or and much more sensitive to chemiluminescence than phosphoimaging systems.

9. REAGENT PREPARATION

Keep all reagents on ice during preparation. Reagents should only be used in their 1X working concentration.

9.1 **1X Wash Buffer I**

Dilute 20X Wash Buffer I 20-fold with distilled or deionized water to prepare the 1X Wash Buffer I.

9.2 **1X Wash Buffer II**

Dilute 20X Wash Buffer II 20-fold with distilled or deionized water to prepare the 1X Wash Buffer II.

9.3 **1X Biotin-Conjugated Anti-Cytokines**

Note: Spin down the tube prior to reconstitution, as the concentrated liquid bead may have moved to the top of the tube during handling.
Add 100 µL 1X Blocking Buffer to the tube containing 2000X Biotin-Conjugated Anti-Cytokines. Mix well and quantitatively transfer stock reagent to larger tube containing 1900 µL of 1X Blocking Buffer. 1X Biotin-Conjugated Anti-Cytokines may be stored for 2-3 days at 2-8°C.

9.4 **1X HRP-Conjugated Streptavidin**

Mix the tube containing 1,000X Streptavidin-HRP well before use, as precipitates may form during storage. Add 2 µL of 1000X Streptavidin-HRP to 1998 µL of 1X Blocking Buffer. This working dilution can be stored for 3-5 days at 2-8°C.

- 1X Wash Buffers can be stored at 2-8°C for up to 1 month.
- Detection Buffers C and D are supplied as 1X solutions that are intended to be mixed in a 1:1 ratio immediately prior use. Detection reagents may be stored at 2-8°C for up to 3 months.

10. SAMPLE PREPARATION AND STORAGE

10.1. General Considerations

- Freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot your samples prior to initial storage.
- Spin samples hard (5-10 minutes at 10,000 to 15,000 RPM) immediately prior to incubation of samples with array.
- Optimal sample concentrations may need to be determined empirically based on the signal intensities of spots and background signals obtained with each sample.
- If spot intensities are weak, increase sample concentration in subsequent experiments.
- If background or spot intensities are too strong, decrease sample concentration in subsequent experiments.
- Most samples will not need to be concentrated. If concentration is required, we recommend using a spin-column concentrator with a chilled centrifuge.

10.2. Recommended Sample Volumes and Dilution Factors

NOTE: All sample dilutions should be made using the 1X Blocking Buffer provided in this kit. For all sample types, final sample volume = 1.0 -1.2 mL per membrane

- **Urine**: 2-fold to 5-fold dilution

  Note: If the sample volume is less than 200 µl, the membrane and sample may be sealed in a small plastic bag to increase sample-membrane coverage. Expel all air bubbles prior to sealing bag.

- **Serum & Plasma**: 2-fold to 5-fold dilution

10.3. **Preparing Urine Samples**

- Prepare 200 µL aliquots and store at –20°C or –80°C as soon as possible after collecting urine samples.
- Addition of protease inhibitors is not required.
- Immediately prior to sample incubation (Step 12.4 of protocol), spin samples at 1,000 rpm for 10 minutes to remove particulates and precipitates.

10.4. **Preparing Serum and Plasma Samples**

- Prepare samples according to established protocols or collection tube manufacturer's instructions. Sub-aliquot into plastic tubes. Store at –20°C or –80°C.
- We do not recommend comparing results between serum and plasma samples or between plasma prepared using different anticoagulants.
- For most applications, you may test plasma samples prepared using any anticoagulant (ie, Heparin, EDTA or Citrate). However, EDTA-prepared plasma may interfere with optimal detection of MMPs and other metal-binding proteins.
- If possible, avoid testing hemolyzed Serum or Plasma samples, as these samples may generate anomalous cytokine expression patterns and/or high background signals.

11. ARRAY MAP

POS – Positive Control
NEG – Negative Control

Array Map for Rat AKI Antibody Array – Membrane C1 (7 Targets) Membrane ab193654

	A	B	C	D	E	F	G	H
1	POS	POS	NEG	NEG	Cystatin C	FABP1	KIM-1	MCP-1
2	POS	POS	NEG	NEG	Cystatin C	FABP1	KIM-1	MCP-1
3	NGAL	TIMP-1	VEGF	NEG	NEG	NEG	NEG	POS
4	NGAL	TIMP-1	VEGF	NEG	NEG	NEG	NEG	POS

Note: L-FABP = Liver Fatty-Acid Binding Protein, KIM-1 = Kidney Injury Molecule-1, NGAL= Neutrophil Gelatinase-Associated Lipocalin (Lipocalin 2).

12. ASSAY PROCEDURE

Please prepare all reagents immediately prior to use. All incubations and washes must be performed under gentle rotation/rocking.

12.1. Place each membrane printed side up into the 8-well tray provided in the kit.

12.2. Block membranes by incubating with 2 mL 1X Blocking Buffer at room temperature (RT) for 30 minutes.

12.3. Aspirate 1X Blocking Buffer from each well. Pipette 1 mL of sample into each well and incubate for 1 - 2 hours at room temperature.

12.4. Aspirate samples from each well. Wash 3 times, 5 minutes per wash, with 2 mL Wash Buffer I at RT. Use fresh buffer for each wash.

NOTE: we strongly recommend an additional step with Large Volume Wash: After step 12.4, place membranes into clean container(s). Add 20-30 mL of Wash Buffer I per membrane, and wash at RT with gentle shaking or rocking for 30-45 minutes. Return membranes to the 8-well tray. Proceed to step 12.5.

12.5. Wash 2 times, 5 minutes per wash, with 2 mL Wash Buffer II each at RT. Use fresh buffer for each wash.

12.6. Pipette 1 mL of 1X Biotin-Conjugated Anti-Cytokines into each membrane and incubate for 1 - 2 hours at RT.

12.7. Aspirate 1X Biotin-Conjugated Anti-Cytokines from each well. Wash membranes as directed in Steps 12.4 and 12.5.

12.8. Pipette 1 mL of 1X HRP-Conjugated Streptavidin into each well and incubate for 2 hours at room temperature.

12.9. Aspirate 1X HRP-Conjugated Streptavidin from each well.

12.10. Wash membranes as directed in Steps 12.4 and 12.5.

12.11. Transfer and place each membrane printed side up onto a plastic sheet (provided in the kit) on your benchtop.

NOTE: Do not allow membranes to dry out during detection. Detection of chemiluminescence should be started within 5 minutes after removing Detection Buffers and must be completed within 20 minutes.

12.12. Remove any excess wash buffer by blotting the membrane edges with another piece of chromatography, blotting, or tissue paper.

12.13. Into a single, clean tube, pipette equal volumes (1:1) of Detection Buffer C and Detection Buffer D. For 1 membrane add 250 µL of Detection Buffer C and 250 µL of Detection Buffer D into tube. Mix well.

12.14. Pipette the mixed Detection Buffers onto each membrane. Place another plastic sheet on top, starting at one end and "rolling" the flexible plastic across the surface to the opposite end. During this process, ensure that the detection mixture completely covers each membrane, and gently smooth out any air bubbles. Avoid sliding the plastic sheet along the membranes' printed surfaces.

12.15. Incubate at RT for 2 minutes.

12.16. Remove top plastic sheet and aspirate excess liquid (see Step 12.12).

12.17. Gently replace the membranes (protein side up) on the bottom plastic sheet and replace the top plastic sheet (see Step 12.14). Gently smooth out any air bubbles on the membrane surfaces.

12.18. Detect signals using a chemiluminescence imaging system or expose the array membranes to x-ray film and develop the film (See tips for obtaining array images in Section 8).

12.19. For each array, use multiple exposures to obtain an image with low background and strong Positive Control signals that do not bleed into one another. Typical exposure times are 10 seconds to 2 minutes.

12.20. When you finish your last exposure, remove the top plastic sheet. Gently rinse membranes and plastic sheets with Wash Buffer II. Remove excess wash buffer as described in

Step 12.14, and replace the membranes between the plastic sheets.

12.21. Wrap the sheets in Saran Wrap, and store the membranes at –20°C to –80°C. (Or store membranes for up to 5 days at 2-8°C in Wash Buffer II. Cover the container to avoid evaporation.)

13. CALCULATIONS

Obtaining Densitometry Data:

Visual comparison of array images may be sufficient to see differences in relative protein expression. However, most researchers will want to perform numerical comparisons of the signal intensities (or more precisely, signal *densities*), using 2-D densitometry. Gel/Blot documentation systems and other chemiluminescent or phosphorescent detection systems are usually sold as a package with compatible densitometry software.

To obtain densitometry data from an X-ray film, one must first scan the film to obtain a digitized image using an ordinary office scanner with resolution of 300 dpi or greater. Any densitometry software should be sufficient to obtain spot signal densities from your scanned images. One such software program, ImageJ, is available for free from the NIH (for more info, visit http://rsbweb.nih.gov/ij/).

We suggest using the following guidelines when extracting densitometry data from our array images:

- For each array membrane, identify a single exposure that the exhibits low background signal intensity and strong Positive Control signals that do not "bleed" into one another. Exposure times do not need to be identical for each array, but Positive Control signals on each image should have similar intensities.
- Measure the density of each spot using a circle that is roughly the size of one of the largest spots. Be sure to use the same circle (area and shape) for measuring the signal densities on every array for which you wish to compare the results.
- For each spot, use the summed signal density across the entire circle (i.e. total signal density per unit area)

Once you have obtained the raw densitometry data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Background Subtraction:

On each array, several “Negative Control” and/or “Blank” spots will be included. Blank spots are literally blank; nothing has been printed there. Negative Control spots are printed with the same buffer used to dilute antibodies printed on the array. Thus, the signal intensities of the Negative Controls represent the background plus non-specific binding to the printed spots. We recommend subtracting the mean of 4 or more Negative Control spots for background correction.

Normalization of Array Data:

The amount of biotin-conjugated IgG protein printed for each Positive Control spot is consistent from array to array. As such the intensity of these Positive Control signals can be used to normalize signal responses for comparison of results across multiple arrays, much like housekeeping genes and proteins are used to normalize results of PCR gels and Western Blots, respectively.

To normalize array data, one array is defined as "reference" to which the other arrays are normalized. This choice can be arbitrary. You can calculate the normalized values as follows:

$$X(Ny) = X(y) * P1/P(y)$$

Where:

P1 = mean signal density of Positive Control spots on reference array

P(y) = mean signal density of Positive Control spots on Array "y"

X(y) = mean signal density for spot "X" on Array for sample "y"

X(Ny) = normalized signal intensity for spot "X" on Array "y"

After normalization to Positive Control signal intensities, you can compare the relative expression levels, analyte-by-analyte, among or between your samples or groups. By comparing these signal intensities, one can determine relative differences in cytokine expression in each sample.

14. TYPICAL DATA

Typical results obtained with Abcam Antibody Arrays:

The preceding figure presents typical images obtained with Abcam Cytokine Antibody Membrane Array. These membranes were probed with conditioned media from two different cell lines. Membranes were exposed to film at room temperature for 1 minute.

Note the strong signals of the Positive Control spots, provided by biotin-conjugated IgG printed directly onto the array membrane in the upper-left and lower-right corners. These Positive Control spots are useful for proper orientation of the array image.

The signal intensity for each antigen-specific antibody spot is proportional to the relative concentration of the antigen in that sample. Comparison of signal intensities for individual antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte sample-to-sample or group-to-group.

15. TROUBLESHOOTING

Problem	Cause	Recommendation
No signal for any spots, including Positive Controls	Complete detection failure	Repeat incubation with HRP-Streptavidin and Detection Buffers.
Weak or no signals antigen-specific spots	Sample is too dilute	Repeat experiment using higher sample concentration.
	Improper dilution of HRP-Streptavidin	Tube may contain precipitants. Repeat detection, mix 1000X HRP-Streptavidin well before diluting reagent.
	Waiting too long to detect chemiluminescent signals.	Repeat detection, making sure to complete this process within 20 minutes.
	Other Tips	Incubate with sample O/N at 2-8°C.
		Increase concentration of HRP-Streptavidin.
		Increase concentration of Biotin-conjugated Anti-Cytokine.
		Extend exposure time (may go overnight).
Uneven signal or background	Bubbles present on membrane during incubations	Be sure to completely remove all bubbles from membrane surface.
	Membranes were not evenly covered during washes/incubations or allowed to dry out	Completely cover membranes with solution, use a rocker or shaker during washes and incubations.
High background signals	Overexposure	Decrease exposure time.
	Sample is too concentrated	Repeat experiment using more dilute sample.
	NOTE: To reduce background on completed membrane, wash overnight at 2-8°C in 1X Wash Buffer II, then re-incubate with HRP-Streptavidin and repeat detection.	

16. NOTES

Technical Support

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