



ITSIARRAY - Antigen - Cat #:K0029-AA

Validated antigen array for screening and rapid detection of auto-antibodies in clinical and biological samples*

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IMPORTANT: K-0029-Ag is a validated kit and procedure to rapidly screen for the presence or absence of auto-antibodies in clinical and experimental samples. This procedure has been extensively tested and successfully applied to human and non-human samples and if carefully followed allows for detection of auto-antibodies against 135 carefully selected antigens. ITSIArray is offered in 3 96 well microtitre plates. It is a unique ELISA based assay that does not require a microtitre plate reader. Each kit which contains ready-to-use reagents and a detection system can be used for cancer, cardiovascular, neuroscience and infectious disease research. Exercise extreme caution when working with proteins and protect your protein sample from breakdown and contamination by wearing gloves and placing tubes on ice. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants such as keratin. **Do not let unused plates to sit at room temperature or in a high moisture environment for more than 1h.**

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT:

Item	Size/Quantity	Catalog #	Storage
ITSIArray-AA 96 Well plate	3	K-0029-AA-P/1-3	-20°C
10X Buffer 1	1 x 500mL	K-0029-AA.1	Rm. T.
Blocker	1 x 20mL	K-0029-AA.3	4°C
Detection Antibody	5 µl	K-0029-AA.4	-20°C
Detection Tablet A and B	3	K-0029-AA.5	-20°C
Stop Solution	1 x 30 ml	K-0029-AA.6	Rm. T.
Procedure			

MATERIALS REQUIRED but Not supplied by ITSI:

1. Biological or Clinical sample.
2. Ice bucket and wet ice
3. Adjustable pipette (Use recently calibrated adjustable pipettes to ensure accuracy)
4. Vortex

PROCEDURE:

- Add the contents of **10X Buffer 1** to 450mL of MiliQ grade water to obtain 500mL of the working buffer. **Note:** The working buffer is used in all steps that require **Buffer 1**.
- Add 20 ml of Buffer 1 to Blocker to prepare the blocking solution.

A. SAMPLE INCUBATION

1. Wash the plate twice with 150 µl of buffer 1 to wet the wells.
2. Dilute your sample 1:1 with buffer 1. (Use dilution only for plasma or serum samples. For cell culture supernatants or other tissue lysates use without dilution).
3. Add 100 µl of the diluted sample to each well and incubate for 2 hour at room temp.
4. Wash the well(s) with 150 µl of buffer 1 and aspirate the liquid. Repeat 2x for a total of 3 washes.

B. DETECTION

5. Add 1 µl of Detection antibody in 20 ml blocking solution to obtain a 1:20,000 solution. To increase sensitivity, a lower dilution can be used.
6. Add 50 µl of the diluted Detection antibody and incubate at room temperature for 1 hour.
7. Wash the wells 4X as in step 4.
8. Bring Detection tablet A and B to room temperature. Add tablets A and B in 5 ml water to prepare the substrate solution and make sure the tablet is completely dissolved. Use within 1h of preparing the solution.
9. Add 100 µl of substrate solution and incubate for 30 mins at room temperature.
10. Add 100 µl of stop solution to each well to stop the reaction. Yellow color indicates positive result. To capture the image, the ITSI Array plate can be scanned on a desktop flatbed scanner. To quantify the color intensity with an ELISA plate reader, read the absorbance at 405nm.

***Conditions for use of this Procedure/Buffers:** This VBP is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream detection step has been validated. Considering that many factors can cause ITSIArray experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and buffers will lead to a successful Protein Array experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the improper use of this procedure or associated materials and reagents.

General Safety Information: Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with water and follow established guidelines/procedures in your laboratory. **Warning: Intended for research use only, not for use in human, therapeutic or diagnostic applications. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**



Stopping Solution (K-0029-AA.6)

Contains Sodium Hydroxide: irritating to eyes and skin; wear suitable protective clothing and gloves; avoid release to the environment.

Distributed Exclusively By:

ITSI-Biosciences, LLC
633 Napoleon Street
Johnstown, PA 15901, USA
Attention: Product Manager

Phone: 1-814-262-7331 Fax: 1-814-262-7334
Website: www.itsibio.com E-mail: itsi@itsibio.com