Protein Microarray

Assay manual and technical recommendations.

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

Sino Biological

1 INTRODUCTION

This manual describes the microarray probing procedure Cy5/Cy3 and compatible fluorophores to detect various antibody isotypes in the serum/plasma samples. In the One-Step detection method, arrays are incubated with anti-human IgG/IgM/IgA that is directly conjugated to the fluorophores. In the Two-Step method, arrays are incubated first with biotinylated anti-human antibody, and then in a second step labeling occurs with Fluorophore-Streptavidin conjugate.

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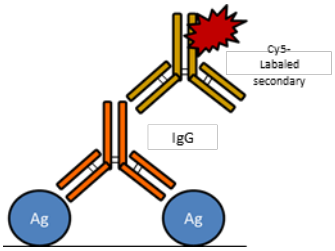
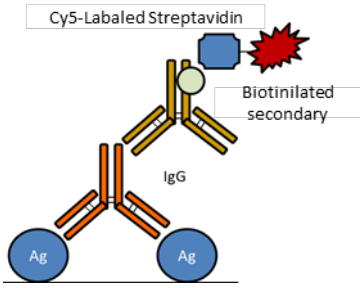
2 OVERVIEW OF PROTEIN MICROARRY

2.1 ONE-STEP AND TWO-STEP DETECTION FOR EACH ANTIBODY ISOTYPE

Single-Step: Detection of antigen-bound IgG by using GAH-IgG-Cy5 as secondary



Two-Step: Detection of antigen-bound IgG by using biotinylated GAH-IgG as secondary antibody and Cy5-streptavidin (SA) conjugate as tertiary.



2.2 STORAGE OF PROTEIN MICROARRAY SLIDES:

Protein Microarray Slides ONCYTE® AVID are custom printed by Nanommune. Store Slides in a light proof box in a desiccator cabinet.

2.3 MATERIALS AND REAGENTS

2.3.1 SUPPLIED

1. Microarray slides
2. *E. coli* Lysate, Lyophilized. Reconstitute each vial in 1 ml of 1x Array Blocking

2.3.2 MICROARRAY ASSEMBLY NOT SUPPLIED

In order to assemble the arrays for probing, you will need the following (all chambers, gaskets, springs and trays are reusable):

1. CHAMBER: ProPlate® Multi-Well Chambers – 16 Well Slide Module. 2 PACK.
   Grace Bio-Labs SKU: 244864

2. TRAY: ProPlate® Tray and Cover for Spring Clip Modules. Holds 3 Slide Modules.
   Grace Bio-Labs SKU: 246879

2.3.3 ASSAY BUFFERS AND REAGENTS NOT SUPPLIED

1. TTBS or COMMERCIAL 10X TTBS: Gentrox Cat # 18-235B, or equivalent**:**
   Filter using 0.2µm Millipore bottle top filter after preparation.

2. 1X ARRAY BLOCKING BUFFER: GVS Cat # 10485356 (1 bottle – 100 ml) - Filter before use and keep at room temperature.

3. ANTIBODIES: Jackson ImmunoResearch or equivalent
   Single-Step: For multiplexing anti-human IgG and IgA
   Alexa Fluor® 647 AffiniPure Goat Anti-Human IgG, Fcγ fragment specific
   (min X Bov, Hrs, Ms Sr Prot). Jackson ImmunoResearch Cat # 109-605-098.

   Alexa Fluor® 488 AffiniPure Goat Anti-Human Serum IgA, α chain specific.
   Jackson ImmunoResearch Cat # 109-545-011;
   or
   Cy™3 AffiniPure Goat Anti-Human Serum IgA, α chain specific.
   Jackson ImmunoResearch Cat # 109-165-011.

   Two-Step: Use biotinylated anti-human IgG or IgA, followed by tertiary Streptavidin
   Biotin-SP (long spacer) AffiniPure Goat Anti-Human IgG, Fcγ fragment specific
   (min X Bov, Ms, Rb Sr Prot)
   Jackson ImmunoResearch Cat # 109-065-170

   Biotin-SP (long spacer) AffiniPure Goat Anti-Human Serum IgA, α chain specific
   Jackson ImmunoResearch Cat # 109-065-011

   Streptavidin: SureLight™ P3
   Columbia Biosciences Cat #: D7-2212

2.3.4 RECOMMENDED EQUIPMENT NOT SUPPLIED

1. Biosafety cabinet
2. Ice bucket
3. Disposable gloves
4. Biohazard waste containers

5. Micropipettes, multichannel micropipette and tips
6. Disposable polypropylene/ polystyrene tubes/ plates for sample preparation
7. Orbital shaker or platform rocker
8. Timer
9. Aspirator: assemble vacuum system using, HEPA filter, Büchner flask, tubing and pipette tips.
10. Ultrapure water
11. Table top centrifuge
12. Microarray Scanner

3 ASSAY PROCEDURE

3.1 TECHNICAL RECOMMENDATIONS

1. Mix reagents by gentle inversion or pipetting before use. Do not vortex or shake reagents to avoid foaming**. Make all reagents homogeneous. Non-homogeneous solutions lead to bias in the signal intensity and overall results.**

2. **Thorough mixing is essential for assay success.** The assay is designed to be run using orbital shakers. Recommended orbital shaking speed is 100-250 rpm.

3. Temperatures or incubation times varying from those specified may give non-reproducible results.

4. Microbial contamination of samples or reagents may produce erroneous results. Turbid reagents or samples should not be used. Avoid multiple sample freeze-thaw cycles. Follow manufacturer`s recommendations for fluorophore reagents storage.

5. Use new pipette tips (autoclaved) for every reagent and washing step, and filtered tips for samples addition step.

6. Ensure complete removal of all solutions and buffers during wash steps, but **NEVER LET PADS DRY** between incubation and wash steps.

7. Plasma, serum, monoclonal antibody, etc. are preferred specimen for use with this test.

8. Serum exhibiting a high degree of hemolysis, lipemia, or microbial growth should not be used as they could yield aberrant results. Specimens containing visible precipitates should be clarified by brief centrifugation prior to testing.

9. Serum may be stored up to one week at 2-10°C. If assay is delayed, serum should be stored frozen at -20°C or lower in a non-self-defrosting refrigerator or freezer. Avoid multiple freeze-thaw cycles.

10. Use of a multichannel pipette is recommended to provide uniform reagent dispensing, incubation times and reaction times.

11. **Avoid touching the microarray pads with pipet tips at all times.**

12. Adequate washing of pads is extremely important. Inadequately washed pads will exhibit high background and potential false positive signals. Completely aspirate liquids when washing and rinsing pads and avoid cross contamination, particularly during the first rinse.

13. Timing of all steps is important. All serum samples and reagents should be prepared prior to beginning of the assay. Batch sizes should be set such that specimen handling can be accomplished comfortably within 5 minutes across all tested microarrays.

14. Follow the recommended storage period indicated for each opened or prepared reagent.

3.2 PROBIMG PROTEIN MICROARRAY

3.2.1 DAY 1: AFTERNOON (duration 40-90 min depending on the number of serum samples)

1. Calculate number of *E.coli* lysate vials needed for probing, and take out appropriate number of vials from desiccator.
    - 1 vial allows probing of 5 slides at 10% blocking.

2. Label on the back side of each microarray slide: microarray type, specimen information, and slide number. Use alcohol resistant marker. Or scan barcodes.

3. Orient labeled microarray slide, pad-side up and small notch at upper right corner. Place ProPlate® chamber over the microarray slide, silicon side down, and secure the slide and chamber with clips. Position the slide/chamber assemblies into a frame. The procedure is illustrated with a 16-pad slide in the diagram below.

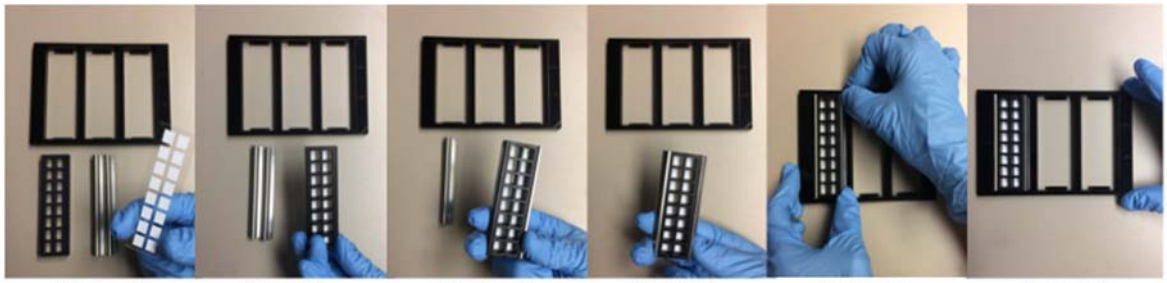


Figure 1. Frame, Chamber, clips and 16-pad slide before assembly
Figure 2. Align the 16-pad slide and the chamber
Figure 3. Snap one clip over one edge of the 16-pad slide/chamber
Figure 4. Snap a second clip over the other edge of the 16-pad slide/chamber
Figure 5. Place the completed 16-pad slide/chamber/clip into the frame
Figure 6. Finished assembly (up to 3 slides can be assembled into the frame)

4. Reconstitute 1 vial of *E.coli* lysate with 1 ml of 1x Array Blocking Buffer. Prepare sufficient volume of 1x Array Blocking Buffer solution containing 10% *E. coli* lysate.
    - i.e., 9 ml 1x Array Blocking Buffer + 1 ml reconstituted *E.coli* lysate to give final concentration of 10% of lysate). Required reaction volume per pad is 100 µl.
    - It is recommended to use 20% *E.coli* lysate for serum with high background

5. Briefly vortex serum or plasma, and centrifuge to pellet any particles (14,000 rpm 30 sec).

6. **In Biological Safety Cabinet:** Aliquot appropriate volume of blocking solution prepared at step 4 into each of the labelled microtubes or wells of 96-well plate. Add serum samples to a final dilution 1:50 to 1:400 depending on serum reactivity. Incubate on rotating platform for 30 min at RT (100-250 rpm). This step blocks any anti-*E.coli* antibodies in the serum.
    - When using mouse serum, *E.coli* lysate is not necessary.
    - Left over reconstituted *E.coli* lysate can be stored at -20°C until next use.

7. Rehydrate assembled microarray pad with 100 ul of 1x Array Blocking Buffer and rotate 30 min at RT (100-250 rpm).

- If there is any leakage, adjust the ProPlate® chambers and clips. Re-apply 1x Array Blocking Buffer and leave at RT 30 min on a rotating platform.

8. Aspirate 1x Array Blocking Buffer from the pads with fine tips (same tip for all pads). Aspirate from the corner of the pad to avoid scratching.
    - Do not allow pads to dry. It may result in high background.

9. Add blocked serum solution (new tip for each serum) to each pad and cover the frame with the lid to prevent evaporation. Place the frame on a moist towel in a tray. Cover the tray with a foil to prevent evaporation, and incubate overnight with gentle agitation (100-250 rpm) on a rocker at 4°C.

Alternate Incubation Method: Incubate as described above 2 hours with agitation (200-250 rpm at RT).

3.2.2. DAY 2: MORNING (Duration about 3h)

10. Aspirate each serum solution with a new pipette tip, wash 3x with 100 ul of TTBS/pad (quick washes). Then perform 3x 5 min washes with **200ul** of TTBS / pad on an orbital shaker at room temperature.

11. If developing arrays with:
    - One-step detection using direct labelled secondary antibody, proceed to **3.2.3**
    - Two-step detection scheme using biotinylated secondary antibody, proceed to **3.2.4**

3.2.3. ONE-STEP DETECTION SCHEME USING DIRECT LABELLED SECONDARY ANTIBODY:

12. Gently mix Fluorophore-conjugated secondary antibody (2° Ab) thoroughly by pipetting before use and always keep the stock vial on ice (Do not vortex). Failure to take homogeneous fractions leads to bias in the signal intensity and overall results. Dilute the desired Fluorophore -conjugated 2° Ab in 1x Array Blocking Buffer to a final dilution of 1:200. Leave the solution on platform rocker to mix for a few minutes at RT.

13. Add 100ul reaction volume of diluted Fluorophore-conjugated 2° Ab to each well and cover with the lid. Incubate **1 hour** with agitation (100-250 rpm) at RT.

3.2.4. TWO-STEP DETECTION SCHEME USING BIOTINYLATED SECONDARY ANTIBODY

14. Gently mix desired biotin-conjugated secondary antibody thoroughly by pipetting before use (Do not vortex). Failure to take homogeneous fractions leads to bias in the signal intensity and overall results. Dilute desired biotin-conjugated 2° Ab in 1x Array Blocking Buffer at 1:400. Leave the solution on platform rocker to mix for a few minutes at RT.

15. Add 100ul of biotin-conjugated 2° Ab to each well and cover with the lid. Incubate **1 hour** with agitation (100-250 rpm) at RT.

16. Aspirate pad, perform 3x 5 min washes with 2x reaction volume of TTBS on an orbital shaker at RT.

17. Dilute Fluorophore-conjugated streptavidin at final dilution of 1:250 in 1x Array Blocking Buffer. Leave the solution on platform rocker to mix for a few minutes at RT.

18. Add 100ul of diluted Fluorophore-conjugated streptavidin to each pad and cover with the lid. Incubate **1 hour** with agitation (100-250 rpm) at RT.

3.3.4. LAST WASHING STEP

19. Aspirate Ab solution from the pads. Perform 3x 5 min washes with 2x reaction volume of TTBS on an orbital shaker at RT.

20. Dissemble microarrays. Avoid scratching pads.

21. Rinse the slides gently with ultrapure water.

22. Place the slides in 50-ml centrifuge tube without caps, and centrifuge 10 min at 500*g* to dry the slides.

23. Store the processed slides in a light proof-box in a desiccator until image acquisition.

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4 REFERENCES

Jain et al., *Evaluation of quantum dot immunofluorescence and digital CMOS imaging system as an alternative to conventional organic fluorescence dyes and laser scanning for quantifying protein microarrays*. Proteomics, 2016. **16**(8): p. 1271-9.