

Protein Array Assay System: QUICK-START GUIDE

The Protein Array Assay System includes the key reagents necessary for maximal use of our ONCYTE[®] nitrocellulose film slides and can be used in various protein microarray applications such as antibody and antigen capture arrays.

Complete User Guide available at: www.gracebio.com/PAAS_userguide

I. Storage and Components

Kit Contents

Store at 4°C

Reagent Box 1 and 2
3× Super G Blocking Buffer (100 ml)
3 × Super G Plus Preservation Buffer (100 ml)
PBST (100 ml)*
PBS (100 ml)*
Protein Array Buffer (10 ml)
Spot Tuning Buffer (1.25 ml)

Notes:

**Due to their high concentrations, these buffers may be most likely to precipitate during shipping/storage. If necessary, follow general recommendations above to re-dissolve buffers prior to using. Once diluted to a 1x working concentration, buffer contents will not precipitate. Do not heat Super G or Super G Plus, keep at 4° C.*

Materials and Reagents Not Provided in this Kit

Materials
ONCYTE [®] porous nitrocellulose film slides
ProPlate [®] Microarray Incubation Chambers

Arraying Reagents
Protease and Phosphatase Inhibitors (see Appendix for recommendations)
Sterile, protease-free water (Sigma W4502, or equivalent)

Assay Reagents
Primary and Secondary Antibodies
Sterile, protease-free water (Sigma W4502, or equivalent)
BSA (Sigma A4737, or equivalent)

For Research Use Only

II. General Recommendations for Array Printing

GBL Protein Arraying Buffer is supplied as a 10x concentrated solution and requires dilution to a 1x working concentration with protease-free water prior to use. For optimal results, proteins should be reconstituted in 1x GBL Protein Arraying Buffer directly from lyophilized proteins.

For applications requiring larger spot sizes, there are multiple methods available to increase the diameter further. Spot Tuning Buffer was designed to provide increased spot size of up to 150% over the non-supplemented buffer.

(See user guide for complete instructions.)

General Recommendations for Printing and Handling of Slides:

- Do not pre-treat ONCYTE[®] porous nitrocellulose film slides prior to spotting.
- Humidity control (if available) set to 50 – 60%.
- Sample plate lids can be utilized during spotting (if possible with your spotter) to minimize sample evaporation.
- Sample cooling with a chilled plate holder is recommended (if possible with your spotter) to minimize sample evaporation and minimize protein degradation.
- Pre-incubate slides in the spotter at desired humidity for 1 hour prior to printing.
- Allow printed arrays to sit in humidified spotter for 1 hour post-printing.
- Store slides in the slide box provided (light blue GBL box). Other slide boxes and trays may contain materials which can off-gas and significantly impact microarray results.
- Store slides overnight at 4°C to maximize protein binding before use. Post-spotting incubation may vary for different proteins and should be tested for specific applications.
- For long-term storage, store slides desiccated at -20°C or -80°C.
- For optimal results, slides should be treated with the supplied Super G Plus Preservation Buffer prior to storage (see next section).

III. Assay Protocols

A. Antibody Capture Assay:

1. Prior to beginning immunoassays, slides should already be blocked/preserved with Super G Plus or Super G. As per those protocols, slides should be washed 3 x 5 min with PBST with agitation (50 rpm).
2. If using a ProPlate chamber fill with appropriate volume of 1x PBST.
3. Incubate with sample containing antigen sample with agitation on an orbital shaker (50 rpm), for 15-60 minutes.
4. Wash with 1x PBST (5 min.) with agitation (50 rpm).
5. Wash with 1x Super G (5 min.) with agitation (50 rpm).

6. Wash with 1x PBST (5 min.) with agitation (50 rpm).
7. Incubate with detection antibody or antibody cocktail in Incubation Buffer with agitation on an orbital shaker (50 rpm), typically 15-60 minutes.
8. Wash with 1x PBST (5 min.) with agitation (50 rpm).
9. Wash with 1x Super G (5 min.) with agitation (50 rpm).
10. Wash with 1x PBST (5 min.) with agitation (50 rpm).
11. Incubate with fluorescently- or biotin-labeled secondary antibody in Incubation Buffer with agitation on an orbital shaker (50 rpm). Typically 15-60 minutes.
12. Wash with 1x PBST (3 x 5 min. with buffer changes) with agitation (50 rpm).
13. *OPTION*: Depending on the detection method where the secondary antibody was biotinylated, perform additional assay amplification incubations such as streptavidin-HRP conjugation, Tyramide Signal Amplification, etc.
 - a. Wash with 1x PBST (2 x 5 min. with buffer change) between steps.
 - b. After final incubation, wash with 1 x PBST (3 x 5 min).
14. Wash with 1x PBS (3 x 5 min. with buffer changes) with agitation (50 rpm).
15. Rinse with fresh deionized water (1 min) with agitation (50 rpm).
16. Allow slides to dry at room temperature for 10 min. prior to data acquisition.

B. Antigen Capture Assay:

1. Prior to beginning immunoassays, slides should already be blocked/preserved as per previous sections with Super G Plus or Super G. As per those protocols, slides should be washed 3 x 5 min with PBST with agitation (50 rpm).
2. If using a ProPlate[®] chamber fill with appropriate volume of 1x PBST.
3. Incubate with serum (e.g. for autoimmune antibody detection) with agitation on an orbital shaker (50 rpm). Typically 15-60 minutes.
4. Wash with 1x PBST (5 min.) with agitation (50 rpm).
5. Wash with 1x Super G (5 min.) with agitation (50 rpm).
6. Wash with 1x PBST (5 min.) with agitation (50 rpm).
7. Incubate with fluorescently- or biotin-labeled secondary antibody in Incubation Buffer with agitation on an orbital shaker (50 rpm). Typically 15-60 minutes.
8. Wash with 1x PBST (3 x 5 min. with buffer changes) with agitation (50 rpm).
9. *OPTION*: Depending on the detection method where the secondary antibody was biotinylated, perform additional assay amplification incubations such as streptavidin-HRP conjugation, Tyramide Signal Amplification, etc.
 - a. Wash with 1x PBST (2 x 5 min. with buffer change) between steps.
 - b. After final incubation, wash with 1 x PBST (3 x 5 min).
10. Wash with 1x PBS (3 x 5 min. with buffer changes) with agitation (50 rpm).
11. Rinse with fresh deionized water (1 min) with agitation (50 rpm).
12. Allow slides to dry at room temperature for 10 min. prior to data acquisition.

Appendices

Appendix A – Protease and Phosphatase Inhibitors

Inhibitor cocktails may also be purchased from commercial sources. Two recommended inhibitor cocktails are listed below. Working concentrations are 1 tablet per 10 ml Arraying Buffer volume.

Complete[™] Mini Protease Inhibitor Cocktail tablet
(Roche Applied Sciences, Cat. # 11 836 153 001)

PhosSTOP[™] Phosphatase Inhibitor Cocktail
(Roche Applied Sciences, Cat. # 04 906 837 001)

Appendix B – Working Reagent Formulas:

GBL Protein Arraying Buffer

Supplied as a 10x concentrated solution. Needs to be diluted with water or available sample buffer with or without additional inhibitors.

Spot Tuning Buffer

Supplied as a concentrated solution. Mix with 1x GBL Protein Arraying Buffer as needed for desired spot size. See Table 1.

Super G Blocking Buffer

Supplied as a 1x solution, ready to use.

Super G Plus Preservation Buffer

Supplied as a 1x solution, ready to use.

1x PBST

(Diluted from 10x concentrated solution)

10 mM Sodium Phosphate, pH 7.4

150 mM Sodium Chloride

0.1 % Tween-20

1x PBS

(Diluted from 10x concentrated solution)

10 mM Sodium Phosphate, pH 7.4

150 mM Sodium Chloride