# Antibody Microarray

# User's Guide

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## INTRODUCTION

Antibody Microarrays provide a high-throughput platform for sensitive, efficient and accurate protein expression profiling, screening, and comparison between normal, diseased or treated samples. Full Moon BioSystems' antibody arrays allow researchers to detect and analyze hundreds of proteins simultaneously on a single slide, saving precious resources and reducing the number of variables that affect experimental outcome. Our unique collection of antibody arrays includes phospho-specific arrays for studying phosphorylation events in cells and tissues, comprehensive exploratory arrays that allow investigators to examine hundreds of proteins in a single experiment, and pathway arrays that are designed for researchers to study highly relevant proteins in their specific research fields. Proteins from cell extracts, fresh, frozen and FFPE tissues, or serum samples can be used for analysis.

## ANTIBODY ARRAYS

The antibodies are covalently immobilized on high quality glass surface coated with our proprietary 3-D polymer materials to ensure high binding efficiency and specificity. All arrays are printed on standard-size microscope slides. The arrays utilize fluorescent detection and can be scanned on all microarray scanners that are compatible with 76 x 25 x 1 mm (3 inch x 1 inch x 1 mm) slides.

For pathway arrays, each slide consists of an array of well-characterized antibodies with six replicates to maximize data reliability. For Explorer Antibody Array, Phospho Explorer Array, Phospho Explorer S1 Array, the antibodies are printed in duplicates.

To see a list of the antibodies featured in a specific array and their reactivity information, please visit our website, www.fullmoonbiosystems.com and select the array of your choice. Go to the Technical Information section to view the Antibody List and Array Layout.

Multiple positive markers and negative controls are included in each array. Positive markers contain Cy3 labeled antibodies to mark the boundaries of the array. Negative controls contain BSA. Empty spots contain nothing and can be used as background in data analysis.

Each set of antibody arrays contains two slides (two identical arrays) – one slide can be used for a control sample, and the other for a treated sample. GAL files are provided for each array and can be downloaded from www.fullmoonbiosystems.com/Support/gal.htm.

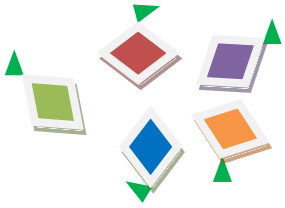
## ANTIBODY ARRAY ASSAY KIT

The Antibody Array Assay Kit is designed for easy and reliable processing of Full Moon BioSystems' antibody arrays. It provides the major reagents required to perform protein extraction, labeling, conjugation and detection. The reagents are convenient, easy to use, and optimized to work with our antibody arrays. Each kit provides sufficient reagents to perform two assays on two slides.

## HOW IT WORKS

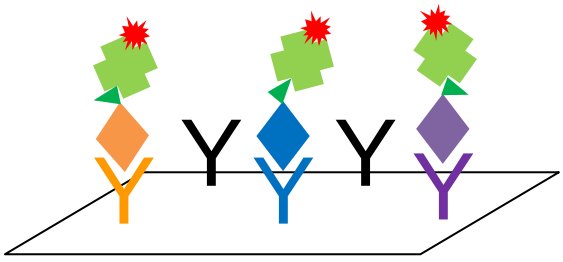
Protein Extraction from cells, tissues, or bodily fluids

Biotinylation of Proteins



Protein Conjugation to Antibody Array

Detection by Cy3-Streptavidin



## EXPERIMENTAL CONSIDERATIONS

- All reagents and materials are intended for research use only.
- Always wear gloves before handling any reagents.
- Handle the slides by holding the area with barcode labels. Do not touch the slide surface.
- Use extra care. Any variation in buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- Only use reagents and materials recommended by this user's guide. Do not substitute buffers or solutions from other sources.
- Do not allow the arrays dry out between blocking, coupling and washes. It can cause high background.
- Wash the arrays extensively with Wash Solutions and water. It helps remove excess residual reagents from the slide surface.
- The reagents provided in the Antibody Array Assay Kit do not contain protease inhibitors. To prevent protein degradation, once you start the extraction, you should work quickly and proceed diligently towards the array analysis step. Alternatively, you may use inhibitors if you prefer or plan to store the proteins for a week or longer.

## COMPONENTS

### Antibody Arrays

| Material/Reagent | Quantity | Purpose | Storage Condition |
|---|---|---|---|
| Antibody Microarray | 2 slides | Microarray | 4°C (6 months) |
| Gal File | 1 | Data Analysis | Online download |
| User's Guide | 1 | Instructions | Online download |

## Antibody Array Assay Kit
(sold separately from the arrays)

| Catalog No. | Description | Quantity |
|---|---|---|
| KAS02 | Antibody Array Assay Kit, 2 Reactions | 2 Reactions |
| KAS20 | Antibody Array Assay Kit, 20 Reactions | 20 Reactions |

| Material/Reagent | Quantity | | Purpose | Storage Condition |
|---|---|---|---|---|
| | 2-Rxn Kit | 20-Rxn Kit | | |
| Biotin Reagent | 1 mg | 5 x 1mg | Labeling | -20 °C |
| Blocking Reagent | 60 mL | 600 mL | Blocking | 4 °C |
| Coupling Chamber | 1 | 5 | Coupling | RT |
| Coupling Reagent | 12 mL | 120 mL | Coupling | -20 °C |
| Detection Buffer | 60 mL | 600 mL | Detection | 4 °C |
| DMF | 200 uL | 1 mL | Labeling | 4 °C |
| Dry Milk | 1.8g & 0.36g | 18g & 3.6g | Coupling | 4 °C |
| Extraction Buffer | 1.5 mL | 15 mL | Cell and tissue lysis | 4 °C |
| Labeling Buffer | 2 mL | 20 mL | Labeling | 4 °C |
| Lysis Beads | 2 tubes | 20 tubes | Cell and tissue lysis | RT or 4 °C |
| Spin Columns | 2 sets | 20 sets | Buffer Exchange | RT |
| Stop Reagent | 100 uL | 1 mL | Stop labeling reaction | 4 °C |
| 10X Wash Buffer | 100 mL | 500 mL x 2 | Washing | 4 °C |

## ADDITIONAL MATERIALS REQUIRED

- 50-ml conical tube with cap
- 1X PBS (pH=7.4)
- Cy3-Streptavidin (e.g. GE Healthcare, PA43001; Invitrogen, S11224, Sigma-Aldrich, S6402)
- Centrifuge
- Compressed nitrogen/clean air. **Do not use canned air duster.**
- Microarray scanner compatible with 3 x 1 inch (76 x 25 mm) slides
- Milli-Q Grade Water or dd $H_2O$
- Orbital shaker
- Petri dishes, 100 x 15mm (9 cm in diameter)
- Spectrophotometer
- Vortexer

## REAGENT PREPARATION

**WARMED REAGENTS BEFORE USE**

| | |
|---|---|
| **Blocking Reagent**<br>**Coupling Reagent**<br>**Wash Buffer** | **Warm to 25-30°C in a water bath.** |
| **Biotin**<br>**Detection Buffer**<br>**DMF**<br>**Dry Milk**<br>**Labeling Buffer**<br>**Stop Reagent** | **Warm to room temperature.** |

**2-RXN Kit (KAS02)**

| | | |
|---|---|---|
| 1 | 1X Wash Solution | Make 1:10 dilution. In a one-liter reagent bottle, add 100 ml of 10X Wash Buffer to 900 ml of dd $H_2O$. Shake to mix. |
| 2 | Blocking Solution | Add 1.8 g of Dry Milk to 60 ml of Blocking Reagent. Shake to mix. Be sure the milk powder is completely dissolved. **Use within one week.** |
| 3 | Coupling Solution | Add 0.36 g of Dry Milk to 12 ml of Coupling Reagent. Shake to mix. Be sure the milk powder is completely dissolved. **Use within one week.** |

**20-RXN Kit (KAS20)**

| | | |
|---|---|---|
| 1 | 1X Wash Solution | Make 1:10 dilution. For example, add 100 ml of 10X Wash Buffer to 900 ml of dd$H_2O$ to make 1L of 1X Wash Solution. Shake to mix. |
| 2 | Blocking Solution | If you plan to perform 20 assays within one week, add 18 g of Dry Milk to 600 ml of Blocking Reagent. Be sure the milk powder is completely dissolved. For two assays, aliquot 60 ml of Blocking Reagent and add 1.8 g of Dry Milk. Shake to mix. **Use within one week.** |
| 3 | Coupling Solution | If you plan to perform 20 assays within one week, add 3.6 g of Dry Milk to 120 ml of Coupling Reagent. Be sure the milk powder is completely dissolved. For two assays, aliquot 12 ml of Coupling Reagent and add 0.36 g of Dry Milk. Shake to mix. **Use within one week.** |

# PROTOCOL – DETECTION BY CY3-STREPTAVIDIN

**IMPORTANT – WARM REAGENTS BEFORE USE**

(See Reagent Preparation for detailed instructions)

## A. Protein Extraction

**Note**: It is highly recommended that protein extraction is performed with the Extraction Buffer provided in the Antibody Array Assay Kit (KAS02). If you plan to use extraction or lysis buffers by other manufacturers, please be sure the buffer is free of Tris or other amine based compounds and its detergent concentration is less than 0.2%. The presence of such compounds adversely affects biotinylation of protein samples in the next step. If such buffer was used to extract proteins from cells or tissues, do not omit Step B (Lysate Purification/Buffer Exchange). This step removes the unwanted buffers from your protein extract and replaces it with the Labeling Buffer provided in the Antibody Array Assay Kit.

**Note**: The reagents provided in the Antibody Array Assay Kit do not contain protease or phosphotase inhibitors. To prevent protein degradation, once you start the extraction, you should work quickly and proceed diligently towards the array analysis step. Alternatively, you may use inhibitors if you prefer or plan to store the proteins for a long period of time.

A. Cells

1. Wash the cell culture with cold 1X PBS (4°C). Collect 1 to 5 million cells by scraping the cells from the plate. Place the cells in a microcentrifuge tube. Remove culture media from the cells. Wash with cold 1X PBS (4°C) three times. Centrifuge at 4°C. Aspirate and discard supernatant.

   **Important**: only use PBS to wash the media/cells. To protect protein activity, avoid using trypsin or other reagents.

2. Add one tube of Lysis Beads to the cell pellet and add Extraction Buffer. The amount of Extraction Buffer should be determined based on the number of cells harvested. For 1 – 2.5 million cells, add 100 ul of Extraction Buffer; for 2.5 – 5 million cells, add 200 ul of Extraction Buffer. Mix rigorously by vortexing for 30 seconds to 1 minute. Incubate the mixture on ice for 10 minutes.
3. Repeat vortexing for 30 seconds to 1 minute at 10-minute intervals for 40 – 60 minutes. Be sure to incubate the mixture on ice between vortexing.
4. Centrifuge the mixture at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C.
5. Transfer the clear supernatant to a clean tube. Discard the beads.

   **Important:** be sure to only aspirate the clear layer of supernatant on the top. If the supernatant appears to be cloudy, transfer the supernatant to a clean tube, centrifuge again at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C. If the supernatant is still not clear, store the lysate at -70°C for 10 to 20 minutes. Remove from the freezer,

immediately centrifuge at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C. Save the top clear layer and discard the rest. The supernatant should look clear and transparent as water.

6. Proceed immediately to Step B (Lysate Purification).

B. Tissues

1. Wash the tissues with cold 1X PBS (4°C) 3 to 5 times with vortexing to remove blood from the tissues.

   **Important**: this step is very critical. Make sure all the blood is removed from the tissues. Increase the number of washes with cold PBS if necessary.
2. Add one tube of lysis beads to 10 – 40 mg of tissues.
3. Add Extraction Buffer to the tissues. The amount of Extraction Buffer should be determined based on the amount of tissues harvested. For 10 – 20 mg of tissues, add 100 ul of Extraction Buffer; for 20 – 40 mg of tissues, add 200 ul of Extraction Buffer.
4. Mix rigorously by vortexing for 30 seconds to 1 minute. Incubate the mixture on ice for 10 minutes.
5. Repeat vortexing for 30 seconds to 1 minute at 10-minute intervals for 40 – 60 minutes. Be sure to incubate the mixture on ice between vortexing.
6. Centrifuge the mixture at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C.
7. Transfer the clear supernatant to a clean tube. Discard the beads.

   **Important:** be sure to only aspirate the clear layer of supernatant on the top. If the supernatant appears to be cloudy, transfer the supernatant to a clean tube, centrifuge again at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C. If the supernatant is still not clear, store the lysate at -70°C for 2 to 4 hours. Remove from the freezer, centrifuge at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C. Save the top clear layer and discard the rest. The supernatant should look clear and transparent as water.
8. Proceed immediately to Step B (Lysate Purification).

C. Serum – Proceed directly to Step C (Protein Labeling).

**B. Buffer Exchange/Lysate Purification**

**Important:** This step ensures the removal of unwanted buffer from your protein extract and replaces it with the Labeling Buffer provided in the Antibody Array Assay Kit.

1. The sample volume capacity of each spin column is 100uL.
2. Gently tap the columns to ensure that the dry gel has settled to the bottom of the column. Remove the top column cap and reconstitute the column by adding 650 uL of Labeling Buffer.
3. Replace the column cap and vortex vigorously for about 5 seconds. Remove air bubbles by sharply tapping the bottom of the column. Allow at least 30 to 60 minutes of room temperature hydration time before using the column.

   **Note**: if the column was stored at 4°C, allow the column to reach to room temperature

before use.

4. After hydration, remove the top column cap and then remove the column end stoppers from the bottom.
5. Spin the column in its wash tubes at 750 x g for two minutes to remove excess fluid.
6. Blot excess drops from the bottom of the column. Discard the wash tubes and the excess fluid. Do not allow the gel material to dry excessively. Process the samples within the next few minutes.
7. Hold the column up to the light. Transfer up to100uL of protein extract, from cells, tissues or bodily fluid, to the top of the gel of each column. Carefully dispense the sample directly onto the center of the gel bed at the top of the column without disturbing the gel surface. Do not touch the sides of the columns with the reaction mixture or the sample pipet tip since this can reduce the purification efficiency.
8. Place the column into a collect tube and place both together into the rotor. Maintain proper column orientation.
9. Spin the column and collection tube at the 750 x g for 2 minutes.
10. The purified protein will collect at the bottom of the collection tube. Discard the spin column.
11. Proceed immediately to the next step.

### C. Lysate quantification and QC

1. Measure the UV absorption of the protein sample.

   **Note**: The absorbance should be greater than 4 OD. If the concentration is too low, the proteins must be concentrated at 4°C in a vacuum centrifuge, such as SpeedVac, or using YM-10 filters (Millipore Corporation).

   **Note**: Two separate peaks should be observed at 200-230nm and 240-280nm. If the peaks are not well separated, it indicates the lysate is not clear enough. To improve the lysate's quality, store the lysate at -70°C for 10 to 20 minutes. Remove from the freezer, immediately centrifuge at 10,000 x g (14000 rpm) for 15 to 20 minutes at 4°C. Save the top clear layer and discard the rest.

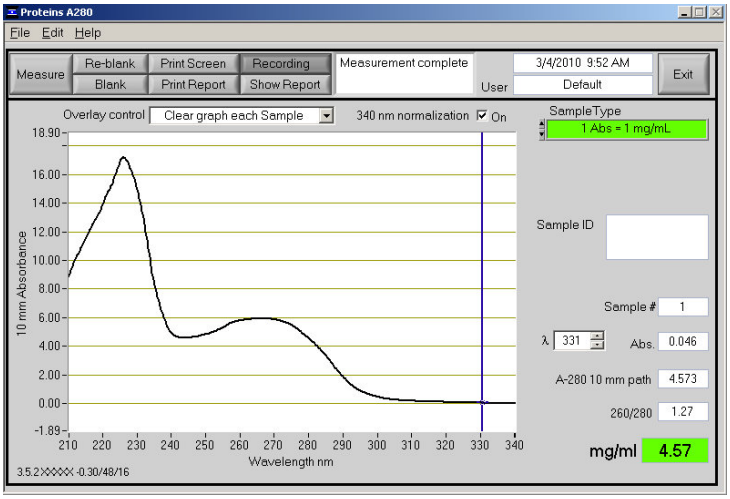



Figure 1. UV absorption spectrum of a clear cell lysate. One peak is observed at 230nm, and a second peak is observed at 270nm. The two peaks are clearly separated. This shows that the lysate is clear and ready for the next step.

2. Proceed immediately to the next step (Protein Labeling) or store the lysate at -80°C.

### D. Protein Labeling – Biotinylation of Protein Samples

1. Biotin Preparation
   a. Briefly centrifuge Biotin Reagent before use.
   b. Add 100 ul of DMF (*N,N*-Dimethylformamide) to 1 mg of Biotin Reagent to give a concentration of 10 ug/ul. Label this solution as Biotin/DMF.
2. Labeling
   a. Aliquot 10 – 25 ul of the lysate. It should contain 80 – 150 OD of protein (or 40 – 100 ug with a concentration of 2 – 10 ug/ul). If you are working serum, aliquot 3 – 4 ul of serum.
   b. Add Labeling Buffer to the protein sample to bring the volume to 75 ul.
   c. Add 3 ul of the Biotin/DMF solution to the protein sample. Incubate the mixture at room temperature for 1 – 2 hours with mixing.

      **Note**: Store the rest of the Biotin/DMF solution at -20°C for future use.
   d. Add 35 ul of Stop Reagent. Incubate for 30 minutes at room temperature with mixing.
   e. Proceed immediately to the next step, or store the sample at -80°C for future use.

### E. Blocking

**Pre-blocking preparation**: Remove the Antibody Microarray from the refrigerator. Before opening the package, allow the slides to warm up to room temperature (30 to 45 minutes). Open the package to expose the slides and allow to dry for 10 to 15 minutes.

1. Add 30 ml of Blocking Solution (See "Reagent Preparation") in a 100x15 mm Petri dish. **Important**: be sure the solution is at room temperature before use.
2. Submerge one slide in the Blocking Solution. The side with a barcode label must face up.
3. Incubate on an orbital shaker rotating at 55 rpm for 30 to 45 minutes at room temperature.
4. Rinse the slide **extensively** with Milli-Q grade water as follows:
   1. Place the slide in a 50-ml conical tube. Fill the tube with 45 ml of water. Close the cap. Shake for 10 seconds. Discard the water.
   2. Repeat ten times.

   **Important**: It is critical to rinse the slide extensively to completely remove Blocking Solution from the slide surface. After the last rinse cycle, the layer of water left on the slide surface should appear uniformly smooth across the entire surface. If it looks spotty, it means the surface is not clean. Please repeat Step 5.
5. Shake off excessive water on the slide surface. Proceed immediately to the next step.

   **Note:** do not allow the slide to dry out. If you are not ready to start coupling, place the slide back in the conical tube filled with clean water.

### F. Coupling

**Note:** You can prepare the Protein Coupling Mix (Step F.1 and F.2) in advance so that you can start coupling immediately after blocking.

1. In a tube, add 6 ml of Coupling Solution (See "Reagent Preparation"). **Important**: be sure the solution is at room temperature before use.
2. Add one tube of biotin labeled proteins (80 – 150 OD or 40 – 100 ug). Vortex briefly to mix. Label it as "Protein Coupling Mix."
3. Place the slide in Well 1 (or any clean well) of the Coupling Chamber.
4. Slowly pour all 6 ml of Protein Coupling Mix over the slide. **Important**: Make sure the slide is completely submerged. Cover the Coupling Chamber.
5. Incubate on an orbital shaker rotating at 35 rpm for 1 – 2 hours at room temperature.
6. Transfer the slide to a 100x15 mm Petri dish containing 30 ml of 1X Wash Solution (See "Reagent Preparation").
7. Incubate on an orbital shaker rotating at 55 rpm for 10 minutes at room temperature. Discard the wash solution. Repeat the wash step twice.
8. Rinse the slide **extensively** with Milli-Q grade water as follows:
    1. Place the slide in a 50-ml conical tube. Fill the tube with 45 ml of water. Close the cap. Shake for 10 seconds. Discard the water.
    2. Repeat ten times.

    **Important**: It is critical to rinse the slide extensively to completely remove Coupling Solution from the slide surface. After the last rinse cycle, the layer of water left on the slide surface should appear uniformly smooth across the entire surface. If it looks spotty, it means the surface is not clean. Please repeat Step 8.
9. Shake off excessive water on the slide surface and proceed to the next step immediately. **Note:** do not allow the slide to dry out.

### G. Detection

1. Add 60 ul of Cy3-Streptavidin (0.5 mg/ml) to 60 ml of Detection Buffer.
2. Pour 30 ml of Cy3-Streptavidn Solution into a 100x15 mm Petri dish.
3. Submerge the slide in the Cy3-Streptavidin solution. Incubate on an orbital shaker rotating at 55 rpm for 20 minutes at room temperature in the dark or covered with aluminum foil.
4. Transfer the slide to a new 100x15 mm Petri dish containing 30 ml of 1X Wash Solution.
5. Incubate on an orbital shaker set at 55 rpm for 10 minutes at room temperature. Discard the wash solution. Repeat the wash step twice.
6. Rinse the slide **extensively** with Milli-Q grade water as follows:
    1. Place the slide in a 50-ml conical tube. Fill the tube with 45 ml of water. Close the cap. Shake for 10 seconds. Discard the water.
    2. Repeat ten times.

    **Important**: It is critical to rinse the slide extensively to completely remove Detection

Solution from the slide surface. After the last rinse cycle, the layer of water left on the slide surface should appear uniformly smooth across the entire surface. If it looks spotty, it means the surface is not clean. Please repeat Step 6.

7. Hold the slide with your fingers, shake off excess water from the slide.
8. Dry the slide with compressed nitrogen (or air) or by centrifugation.

   **Note**: The goal is to remove water from the slide as quickly as possible. Do not use compressed air in a can (for example, desktop air duster). Compressed air or nitrogen from a cylinder tank or an outlet on the fume hood is adequate. Make sure the pressure is less than 30 psi. Point the air nozzle at a 30° angle, one-half inch away from the slide surface. Starting from one end of the slide, push the water off of the surface. Repeat for the back side of the slide.
9. The slide is now ready for scanning.

   Note: If you do not have access to a microarray scanner, you can send the slides to our lab for scanning. For more details, please visit our website:

   http://www.fullmoonbiosystems.com/Services/ArrayScanning.htm.

   To prepare the slides for shipping, place the slides back in the slide holder. Cover the slide holder with aluminum foil to protect the slides from light. Send the package at room temperature. Please include the array information and your contact information (name, organization, phone and email address) in the package.

   Shipping address:

   Attn: Array Scanning Service
   Full Moon BioSystems, Inc.
   754 North Pastoria Avenue
   Sunnyvale, CA 94085
   United States
   Phone: 408-737-1702
   Email: support@fullmoonbiosystems.com

## ARRAY SCANNER RECOMMENDATIONS

All scanners that are compatible with 75mm x 25mm (3in x 1in) microscope slides can be used to scan Full Moon BioSystems' antibody arrays.

Recommended Scanning Resolution: 20um or higher (10um, 5um, etc.)

### Selected Compatible Systems

| Manufacturer | Product Name | Required Accessory |
|---|---|---|
| Agilent Technologies | DNA Microarray Scanner | |
| Alpha Innotech | AlphaScan™ Microarray Scanner<br>NovaRay™ Detection Platform | |
| Applied Precision | arrayWoRx®e Biochip Reader | |
| Aurora Photonics | PortArray 5000™ | |
| Biomedical Photometrics | The DNAscope™ HR | |
| Eppendorf North America | Eppendorf Silverquant® scanner system | |
| GE Healthcare, formerly Amersham Biosciences | Typhoon™ 9410, 9210, 8610 | Microarray Slide Holder Kit |
| Genewave | AmpliReader™ 4600 Microarray Reader | |
| INNOPSYS | InnoScan® Microarray Scanner<br>700, 900, Autoloader | |
| Molecular Devices, formerly Axon Instruments | GenePix® Microarray Scanner<br>4000A, 4000B, 4100, 4200L | |
| PerkinsElmer, formerly Packard Bioscience | ProScanArray® HT Microarray Scanner<br>ProScanArray® Microarray Scanner<br>ScanArray® GX Microarray Scanner<br>ScanArray® GX PLUS Microarray Scanner | |
| Tecan | LS Reloaded™ Versatile Scanner<br>PowerScanner™ High Resolution Microarray scanner | |
| Vidar Systems | Revolution™ 4200 Microarray Scanner | |

### Incompatible Systems

| Manufacturer | Product Name |
|---|---|
| Affymetrix | GeneChip® Scanner, HT Array Plate Scanner |
| Illumina | BeadStation 500GX Genetic Analysis System |