Omega Health & Nutrition

Microarray Trouble Shooting Guide

Problem	Possible Cause	Solution
No signal, very weak signal, or dull pads		
	Omission of a key reagent or the reagents were added in the incorrect order.	Check the kit Instructions for Use for the assay protocol and repeat the assay.
	Samples were not diluted in the kit sample diluent or incorrect dilution was used.	Verify that the samples were added to the sample diluent provided in the kit at the correct dilution.
	Incorrect volume of reagents was added to the microarray.	Ensure that the volumes stated in the kit Instructions for Use are adhered to.
	Incubation times inadequate.	Incubation times stated in the Instructions for Use of the kit should be strictly adhered to.
	Laboratory temperature was too low.	Maintain the room temperature within 18 – 25°C. Avoid running assays under air conditioning vents or near cold windows.
	Reagents and microarray slides were too cold.	Ensure all components are brought to room temperature before use by taking them out of the refrigerator, and taking the kit components out of the box, at least 2-3 hours before starting the assay.

No signal, very weak signal, or dull pads (continued)	Wrong conjugate was used or has deteriorated.	Ensure that the conjugate used is the one that was included in the kit as conjugates are kit and lot specific. If decanting into another vessel, ensure that the vessel is clean from contaminants. Ensure that only the amount required for immediate use is poured out and do not return any unused portion to the bottle.
	Incorrect wash solution was used.	Ensure that the wash solution recommended for the kit is used. The wash solution should not be diluted in any way.
	Excess wash buffer was not removed from the microarray slides after washing.	Ensure that excess wash buffer is vigorously flicked out before proceeding to the next step.
	Substrate was no longer active.	Check the colour of the substrate. If coloured blue, activity will be reduced. If decanting into another vessel, ensure that the vessel is clean from contaminants. Ensure that only the amount required for immediate use is poured out and do not return any unused portion to the bottle.
	Wrong substrate used.	Ensure that the substrate supplied in the kit is used in the assay.
	Reagents were expired or intermixed from a different lot number.	Verify the expiration dates and lot numbers on the reagents. Ensure that only kit matched components are used.

No signal, very weak signal, or dull pads (continued)	Excessive kit stress has occurred.	Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left out in extreme temperatures.
	Kit has been open longer than 3 months.	Ensure that a kit is used within 3 months of opening.
	Microarray slides were compromised.	Ensure that slides are refrigerated in the slide box and then in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on slides by allowing them to equilibrate to room temperature while in the packaging.
	Assayed slide was bleached from storage in sunlight.	Ensure that the slide is protected from direct sunlight after assay is complete.
	Incorrect GenePix Pro settings were used.	Verify the settings in the Software User Manual provided on the disc enclosed in the kit.
	Scanner not properly warmed up or too long between turning on and scanning.	Allow scanner to warm up and do scan immediately.
	Slide not completely dry before scanning.	Ensure that the slides are centrifuged after the final wash and are left to dry for at least 30 minutes as described in the kit Instructions for Use.
	Equipment out of calibration.	Check that the pipettes and machines being used are calibrated correctly.
	Operator training.	Ensure that the operator is correctly trained.

Problem	Possible Cause	Solution
Signal too high		
	Reagents were added in the incorrect order.	Check the kit Instructions for Use for the assay protocol and repeat the assay.
	Samples were not diluted in the kit sample diluent or incorrect dilution was used.	Verify that the samples were added to the sample diluent provided in the kit at the correct dilution.
	Incorrect volume of reagents was added to the microarray.	Ensure that the volumes stated in the kit Instructions for Use are adhered to.
	The incubation time for one/all of the incubation steps was too long.	Adhere to the incubation times stated in the Instructions for Use.
	Laboratory temperature was too high.	Maintain the room temperature within 18 – 25°C. Avoid running assays near heat sources or in direct sunlight.
	Inadequate washing or incorrect wash solution used.	Ensure that washing is carried out as described in the kit Instructions for Use. Excess wash buffer should be vigorously flicked out before proceeding to the next step. The wash buffer provided in the kit should be used and should not be diluted in any way.

Signal too high (continued)	Wrong conjugate was used or has been contaminated.	Ensure that the conjugate used is the one that was included in the kit as conjugates are kit and lot specific. If decanting into another vessel, ensure that the vessel is clean from contaminants. Ensure that only the amount required for immediate use is poured out and do not return any unused portion to the bottle.
	Wrong substrate was used or has been contaminated.	Ensure that the substrate used is the one that was included in the kit. Check colour of substrate. If coloured blue, activity will be compromised. If decanting into another vessel, ensure that the vessel is clean from contaminants. Ensure that only the amount required for immediate use is poured out and do not return any unused portion to the bottle.
	Reagents were expired or intermixed from a different lot number.	Verify the expiration dates and lot numbers on the reagents. Ensure that only kit matched components are used.
	Kit has been opened longer than 3 months.	Ensure that a kit is used within 3 months of opening.
	Equipment out of calibration.	Check that the pipettes and machines being used are calibrated correctly.

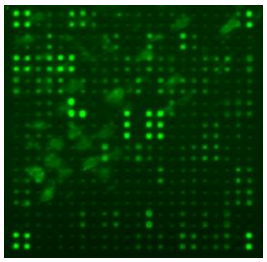
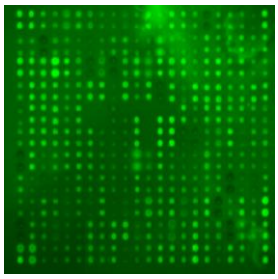
Signal too high (continued)	Dust on the slide.	Ensure that the assay is not carried out in a high traffic area in the laboratory. Do not carry out assay by an open window or air conditioning unit. Do not use standard paper towels to wipe the slide frame (Use lint-free wipes) Ensure that the processing area is as dust free as possible.
	Slide Frame is dirty.	Immerse the disassembled slide frame in alcohol (70%) for < 10 minutes.
	Dust on the scanner unit.	Ensure that the glass scanner bed is clean. Cleaning should be carried out using an anti-static cleaner.
	Incorrect GenePix Pro settings were used.	Verify the settings in the Software User Manual provided on the disc enclosed in the kit.
	Operator training.	Ensure that the operator is correctly trained.
	Slide not completely dry before scanning.	Ensure that the slides are centrifuged after the final wash and are left to dry for at least 30 minutes as described in the kit Instructions for Use.

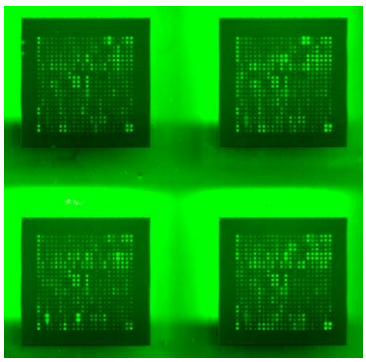
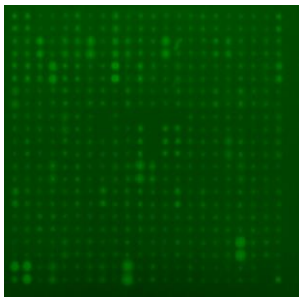
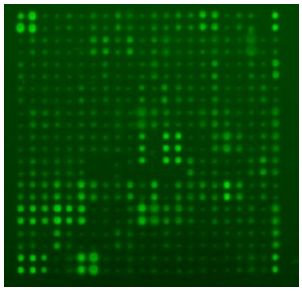
Problem	Possible Cause	Solution
High background		
	Splashing of liquid from positive pads (contamination).	Ensure samples and reagents are added to the assay carefully.
	Reagents were intermixed or contaminated.	Ensure that the correct reagents were used and that contamination has not occurred.
	Samples were diluted in the Conjugate rather than in the Sample Diluent.	Ensure that the samples are diluted in the kit sample diluent at the correct dilution.
	Substrate solution has deteriorated/was contaminated.	Make sure the substrate is colourless prior to addition to the microarray. Use a clean container if decanting substrate prior to addition to the assay. Ensure that only the amount required for immediate use is poured out and do not return any unused portion to the bottle.
	Laboratory temperature was too high or too low.	Maintain the room temperature within 18 – 25°C. Avoid running assays near heat sources, in direct sunlight or under air vents.
	Inadequate washing or incorrect wash solution used.	Ensure that washing is carried out as described in the kit Instructions for Use. Excess wash buffer should be vigorously flicked out before proceeding to the next step. The wash buffer provided in the kit should be used and should not be diluted in any way.

High background (continued)	Slide not completely dry before scanning.	Ensure that the slides are centrifuged after the final wash and are left to dry for at least 30 minutes as described in the kit Instructions for Use.
	Dust on the slide.	Ensure that the assay is not carried out in a high traffic area in the laboratory. Do not carry out assay by an open window or air conditioning unit. Do not use standard paper towels to wipe the slide frame (Use lint-free wipes) Ensure that the processing area is as dust free as possible.
	Dust on the scanner unit.	Ensure that the glass scanner bed is clean. Cleaning should be carried out using an anti-static cleaner.
	Snowy effect over the array caused by inadequate sample preparation.	Ensure that all serum/plasma samples are centrifuged at 14,000g for 10 minutes prior to use, as detailed in the kit Instructions for Use.
	Operator training.	Ensure that the operator is correctly trained.

Problem	Possible Cause	Solution
Poor Reproducibility slide to slide		
	Inconsistent incubation times occurred from slide to slide.	Ensure that each slide tested has consistent incubation periods.
	Inconsistent washing occurred from slide to slide.	Use the same number of washes for each slide.
	Pipette was working incorrectly.	Verify pipette calibration and check that the pipette tips are on tight. Ensure that all channels of the pipette draw and dispense equal volumes.
	Kit components and samples were at different temperatures.	Be sure to allow sufficient time for all kit components and samples to come to room temperature. Larger volumes will require longer equilibration time.
	Reagents were being used from different kit lots.	If running two different kit lots at the same time, make sure to label all components so that all reagents within a lot are used with the corresponding microarray slides.

Problem	Possible Cause	Solution
Small marks on array	Small marks seen on the array can be caused by tip strikes.	Ensure that the pads are not touched with the pipette tips during the assay procedure.
Small marks on array	Gel spheres from gel separation tube.	Ensure that sample is centrifuged.

Small marks on array	Dust	Make assay area as dust free as possible. Use only lint free wipes to wipe the slide frame. Do not carry out assay under air conditioning unit or open window.
Leopard skin print on array 	Leopard skin print on array caused by a fingerprint.	Ensure that the pads are not touched at any point during the assay procedure.
Tide mark on array 	Tide mark seen on the scanned slide caused by slide drying before next reagent step.	Ensure that each step is carried out promptly to avoid drying between steps.
Slide looks “fuzzy” with weak standards	Wrong side of slide scanned.	Ensure that the slides are placed correctly into the scanner, as detailed in the User Manual provided on the disc enclosed in the kit.
Lots of Positive foods on the array.	Slide not dry before scanning.	Ensure that the slides are centrifuged after the final wash and are left to dry for at least 30 minutes as described in the kit Instructions for Use.
Message displayed as follows “data set has been self healed”	Gal file has not been loaded correctly.	Ensure that a gal file is loaded each time an image is processed.

Array very bright, standards over 40K F value 	Wrong settings used in SpotWare.	Verify the settings in the Software User Manual provided on the disc enclosed in the kit.
GAL file will not fit after pressing F8	Wrong .gal file used	Load new .gal file from Software CD
GAL file will not fit after pressing F8	Wrong GenePix settings used	Load new settings from Software CD
GAL file will not fit after pressing F8	Wrong side of slide has been scanned 	Check slide orientation
GAL file will not fit after pressing F8	Slide scanned upside down 	Check slide orientation.