Hundreds of variables influence ELISA data. Below is a collection of general assay running tips and troubleshooting strategies that you may find helpful when trying to resolve ELISA issues. For Q-View Data Analysis tips, please see http://www.quansysbio.com/data-analysis-q-view.

When running a Q-Plex™ Array:

Do's

- DO set up, calibrate, and practice using the Q-View™ imager BEFORE starting the assay.
- DO be exact when calibrating shaker speed, being off by even 100 RPM can affect results
- DO dilute all sample types at least 1:2 (50%) with sample diluent (except cell culture media, which can be tested NEAT)
- DO load all standards and samples into the microplate within 10 minutes of each other
- DO be exact with incubation times, particularly the SHRP incubation
- DO be exact when mixing Substrate A and B, being off by even 50 µL can affect results, and mix thoroughly

Don'ts

- DON'T allow the plate to dry out between steps, particularly between washing SHRP and adding substrate
- DON'T allow the SHRP, substrate, or IR dye to be exposed to UV light, as this may degrade it
- DON'T analyze from a color or jpeg image; save grayscale images using a lossless image file type such as Tiff or RAW

Q-Plex Experimental Troubleshooting

Problem	Probable Cause	Solution or Action
Saturated signal on…	Whole Plate/Standard: Incubation conditions for one or more steps were too long, too warm, or had too high RPM	Decrease incubation time, temperature, or RPM if found to be incorrect (instructions for shaker calibration can be found on our website)
	Whole plate/Standard: Incorrect imager settings: aperture may be too open, exposures too long	Adjust settings, e.g. set aperture (f-stop) to the lowest value possible to a minimum of 2, and reimage immediately
	Whole Plate/Standard: Image has been compressed	Export using higher resolution/bit depth and lossless file type such as Tiff
	Standard only: Standard was reconstituted with less volume than recommended (too concentrated)	Rerun, reconstituting with recommended volume
	Samples only: Analyte concentrations in the samples may be above the limit of quantification or signal may be a false positive due to heterophilic antibodies	Samples may require a greater dilution. Test them at several dilutions, such as 1:2, 1:20, and 1:200

No or dim signal on…	Whole Plate/Standard: Incubation conditions for one or more steps were too short, too cold, or had too low RPM	Increase incubation time, temperature, or RPM if found to be incorrect (instructions for shaker calibration can be found on our website)
	Whole plate/Standard: Incorrect imager settings: aperture may be too closed, exposures too short, poor focus	Adjust settings, e.g. set aperture (f-stop) to the lowest value possible to a minimum of 2, and reimage immediately
	Whole Plate/Standard: Image has been compressed	Export using higher resolution/bit depth and lossless file type such as Tiff
	Whole Plate: One or more steps of the assay protocol was skipped or performed with expired reagents	Check kit expiration. Retest with non-expired reagents, ensuring that all reagents are added to the plate at the appropriate times
	Whole Plate: The SHRP, substrate, or IR dye was exposed to UV light and has degraded	Retest using fresh substrate
	Whole Plate (IR): Spots disappear when image is imported	Ensure that a grayscale image is imported
	Standard Only: Standard was reconstituted with higher volume than recommended (less concentrated)	Reconstitute with the volume recommended in the kit manual
	Samples Only: Analyte concentrations in the samples may be below the limit of quantification for the assay(s)	Samples may require less dilution, Retest using a minimum dilution of 1:2 for serum and plasma, or undiluted for cell culture media and urine. Also inquire about our high sensitivity products/protocols
High well background	Total protein content of samples is too high	Samples may require a greater dilution. Test them at several dilutions, such as 1:2, 1:20, and 1:200
	Plate washing or aspiration is not sufficient	Ensure that all channels in the automatic plate washer or pipette are functioning properly and uniformly
	Sample type is incompatible with the assay	Try a different sample type sample preparation. Inquire about our sample testing or custom assay development services
High spot background	Negative control samples, diluents, or wells were contaminated with positive sample	Retest samples and ensure that no contamination takes place

High variation between replicates, poor precision	Plate washing or aspiration is not uniform	Mask outliers or retest, ensuring that all channels in the automatic plate washer or pipette are functioning properly and uniformly
	Well-to-well contamination	Mask outliers or retest, ensuring that no reagent touches plate seals, pipette tips, etc., between uses
	Reagents, such as samples or Substrate A and B, were not homogenous	Mask outliers or retest, ensuring that reagents are well mixed and that particulate matter is removed by centrifugation before addition to the microplate
	Saliva contamination may cause certain assays in a well to be falsely dim or bright	Mask outliers or retest, wear a face mask during all sample handling, pipetting, and washing steps
	Partial drying between steps	Mask outliers or retest, ensuring that reagents are added immediately after washing; if you cannot load the next reagent right away, leave wash buffer in the wells up to 10 mins
	Pipetting or sampling errors	Ensure that pipettes are calibrated, and samples are thoroughly homogenized and particulate matter has been removed by centrifugation
	Image has poor resolution or has been overly compressed	Ensure that the imager is compatible with the product; don't analyze from a jpeg, only analyze from grayscale Tiff
	Plate overlay in Q-View Software may be misaligned for a well or group of wells	In Q-View, ensure that all overlay spots are over the correct assay spots
Edge Effects: Outside wells different signal than inner wells	Uneven temperature around work spaces	Ensure proper incubation conditions are used
	Plate cover not affixed to edges leading to reagent evaporation	Ensure plate seals are adhered to entire microplate
Drift: Replicates loaded over time differ in signal	Replicates were not loaded within 10 minutes, or reagents were not brought to temperature before being loaded	Samples should be brought to temperature, prepared for loading in advance, then loaded onto the microplate together, generally within 10 minutes

Additional Troubleshooting for IR Q-Plex Kits

Problem	Probable Cause	Solution or Action
Flecks of signal outside of wells	Dust, finger prints, etc.	Clean the bottom of the plate and the imager glass with 70% ethanol
Spots have dim centers	Plate became re-humidified after being dried	If the Licor scanner is not immediately accessible after drying, tightly seal the plate using a plate seal, and keep the plate in the dark
Dim signal for standard curve or whole plate	IR Dye was exposed to ambient light too long	Ensure that the IR dye in the vial and on the plate are kept in the dark as much as possible
All spots disappear when imported	Image is not grayscale	Ensure that a grayscale image is imported

Imaging Troubleshooting

Problem	Probable Cause	Solution or Action
High variation between replicates, poor precision	Image has poor resolution or has been compressed	Ensure that the imager is compatible with the product; don't analyze from a jpeg, only analyze from lossless image file types such as Tiff
Blurry Spots	Camera is out of focus f-stop may be too low	Refocus camera and retake image Set f-stop to a minimum of 2
Rectangular spots or repeating pixel intensity values (e.g. 65535)	Image is saturated or has been compressed	Ensure binning/pixel size is as low as possible; Export using higher resolution/bit depth and lossless file type such as Tiff
Standard curves appear flat at one or both ends	Images are over/under exposed Image has been compressed	Re-image using shorter/longer exposure times Ensure binning/pixel size is as low as possible; Export using higher resolution/bit depth and lossless file type such as Tiff
Recommended exposure time(s) are insufficient to obtain quality standard curves	F-stop or ISO problem Flat-Field correction problem Auto-Stop Exposure is turned on	Adjust to settings recommended in user manual Retake flat-field image (recalibrate the imager) Turn off Auto-Stop feature
Spots disappear when the image is imported into Q-View	Image is not grayscale	Ensure that a grayscale image is imported

We take great care to ensure that our products are suitable for use with all valid samples. If you have any questions about troubleshooting, or about our products or services, please contact us at 888-QUANSYS (782-6797) OR INFO@QUANSYSBIO.COM.