No signal

Possible Causes	What can you do?
Assay set up incorrectly	Make sure that the instructions in the protocol is followed carefully.
Incorrect secondary antibody used	Check if the correct secondary antibody is used.
Insufficient antibodies used	Increase concentration of primary or secondary antibody.
Substrate reagents not fresh	Use fresh substrate reagents
Wrong settings of plate reader	Check the settings (wavelength, filters, gain etc) of plate reader.
Insufficient incubation	Follow the incubation time as indicated in the protocol booklet.
Sample concentration falls below detection limits of kit	Decrease dilution factor or concentrate samples.
Plate washing too vigorous	Check the setting of plate washer. Pipette wash buffer into wells gently.
Wells dried out	Cover plate with adhesive film or incubate in humidified chamber throughout experiment.
Enzyme inhibitor present in buffers or reagents	Inhibitors such as Sodium Azide can affect enzyme and assay performance. Ensure that there is no enzyme inhibitor in any buffers.

Weak signal

Possible Causes	What can you do?
Insufficient coating	Use more antigens or antibodies for coating.
Substrate reagents have expired or prepared at a wrong pH	Use fresh substrate reagents.

Variation among replicates

Possible Causes	What can you do?
Improper washing	Make sure that the washing is done as according to protocol.
Poor mixing of samples	Mix samples gently and evenly.
Dirty plate	Make sure that the bottom of plate is clean.
Reagents too old	Make sure that the reagents are not expired. Use freshly prepared reagents.
Bubbles in wells	Make sure that there is no bubble in wells before reading.
Inconsistent pipetting	Calibrate pipettes to make sure that the correct volume is dispensed.
Edge effects	Make sure that the plate and reagents are equilibrated to room temperature before starting assay.

High background

Possible Causes	What can you do?
Too much antibodies was used	Reduce the concentration of primary or secondary antibodies.
Antibodies bind nonspecifically	Use blocking buffer or choose another affinity-purified antibody.
Too much substrate reagent used	Use substrate with higher dilution.
Insufficient washing	Increase washing cycles.
Wrong concentration of blocking reagent	Check the recommended concentration of blocking buffer.
Reaction not stopped	Stop reactions with STOP buffer before reading.
Plate left too long before reading	Take measurements shortly after addition of substrate and STOP buffer.
Insufficient Tween in wash buffer	Use PBS+0.05% Tween as wash buffer.
Incubation temperature too high	Optimize incubation temperature for each experiment.
Plate stacking during incubation lead to uneven temperature throughout the plate	Avoid stacking plates together during incubation.
Pipetting error	Calibrate pipettes to make sure that the correct volume is dispensed.
Reagents not mixed properly	Make sure that all reagents are mixed properly and equilibrated to room temperature before assay
Salt concentration of incubation and wash buffer	Increase salt concentration to reduce nonspecific interaction.
Substrate incubation carried out in light	Perform substrate incubation in dark.
Dirty plate	Make sure that the bottom of plate is clean.

Poor standard Curve

Possible Causes	What can you do?
Improper standard dilution	Use appropriate diluent as blank. Make sure that the dilution is performed as according to protocol.
Standard improperly reconstituted	Briefly spin standard vial before opening. Make sure that there is no undissolved material after reconstituting.
Standard degraded	Store standards as according to protocol.
Curve doesn't fit the scale	Try plotting log-log or 5 parameter logistic curve fit.
Pipetting error	Calibrate pipettes to make sure that the correct volume is dispensed.
Incomplete washing	Increase washing cycles.