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BioOcean ELISA troubleshoot guide

1) If and when customers report difficulty with their kits, what is the most common difficulty they report? 2) What sort of tips do you generally offer to customers to help overcome these challenges?

1. No signal

Cause	Solution
Sample prepared incorrectly	Make sure the samples are prepared correctly following the instruction
Target below the detection limit level	Concentrate or dilute less of samples
Specificity is wrong	Double check the specificity and the cross-reactivity
Incubation time too short	Incubate for longer time (4-6 hours) or overnight at 4^0C
Buffer incompatibility	Check the buffer in the sample for harsh detergent or salt, dilute sample with assay buffer if needed.
Slow color development due to bad substrate solution	Use freshly made substrate solution. Test the substrate solution with small amount of HRP-conjugated reagent
Over-washed or dried out plate	Reduce the wash times and speed if using plate washer, or cover the plate with seal or lid for shorter time between steps.
Plate reader not set correctly	Check the wavelength used to measure on plate reader.

2. High background

Cause	Solution
Insufficient wash of plate	Increase wash times or cycles. Tamp the plate upside down onto a thick bed of clean paper towels between wash.
Too much detection or HRP-conjugated antibodies	Follow the instruction to use only suggested amount with correction dilution factors.
Contaminated assay buffer	The assay buffer should be prepared in a clean container before transferred.
Blocking was not done correctly	Check the blocking buffer for contamination or ineffectiveness, make sure to add enough blocking buffer to cover most surface in each well.
Light affects substrate	Avoid incubating substrate solution in direct strong light for prolonged time. Keep in dark if possible.

3. Poor standard curve

Cause	Solution
Wrong serial dilution	Re-draw the standard curve with corrected concentration of standards.
Standard reconstituted incorrectly	Spin the standard tube briefly and bring down all contents before adding reconstitute buffer, make sure it is completely dissolved.
Standard expired or degraded	Following the storage condition. After reconstitution, avoid repeat thawing or store in several aliquots.
Pipetting error	Use only calibrated pipettes, and handle with proper techniques.

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4. Low sensitivity

Cause	Solution
ELISA kit was not stored properly or became expired	Follow the instruction to store the kit and use it before expiration date.
Incompatible sample (serum, conditioned media, cell extract)	Detection could be greatly effected if the sample type was not indicated in the instruction. Follow the antigen extraction procedure or include a known positive sample control in the assay.
The kit is not sensitive enough	Many ELISA kits also have high-sensitivity version to detect target with very low abundance. Switch to these products.
Over diluted sample	Use less dilution when prepare samples.
Plate reader not calibrated	Make sure the wavelength to measure is correctly set or calibrated.

5. Large variation

Cause	Solution
Wells were washed unevenly	Use calibrated plate washer to wash or practice consistent way manually. Wash plate gently but thoroughly. DO NOT directly flush the coating surface.
Air bubble in wells	Air bubble easily occurs due to detergents in solution, please double check each well after pipetting or shake the plate to remove air bubble.
Pipetting not consistent	Use calibrated pipette and proper technique, especially when pipetting substrate and stop solution.
Sample not mixed thoroughly	Prepare samples and all reagents by thoroughly mixing.
Unstable environment	Avoid adding too warm or too cold reagent to the plate through the assay.
Plate reader setting wrong with dimension	The reading of plate was not optimized for each well. Double check the plate type in setting.

2) Other tips.

1. Always try to use fresh plate and fresh sample. Collect all samples and store properly in stable condition before running an assay. Maintain good condition and properties of both antibody and target protein is extremely critical.

2. Never use expired product. Keep and store all reagents sterile to prevent microorganism from contaminating the components. Pay attention to store different components of kit at proper condition.

3. Mix samples thoroughly before adding to the plate. Run duplicates or even triplicates for each samples. Some samples need special extraction procedure to gain the best result.

4. Make sure to include all positive AND negative samples and run them all on one plate. If multiple plates need to be used within an experiment, use these plates from the same lot and were stored in the same condition.