

High Background or Excessive Color Development

Possible causes	Recommended actions
Poor-quality water was used to wash plates or to prepare wash solution.	Check the water quality. If it is questionable, try substituting an alternate water source, such as bottled distilled water, to wash plates or prepare the wash solution.
Substrate solution has deteriorated.	Make sure the substrate is colorless prior to addition to the plate.
There was insufficient washing or poor washer performance.	Try using the highest number of washes recommended for the assay. Make sure that at least 350 μ L of wash solution is dispensed per well per wash. Verify the performance of the washer system. Have the system repaired if any ports drip, dispense or aspirate poorly.
Washer system had microbial contamination.	Clean out microbial contamination by flushing the system with a dilute solution of bleach (10% by volume) followed by a large amount of distilled or deionized water. Prime the system with the appropriate wash solution before use. The tubing may need to be changed if the contamination is heavy.
Wash system contained an alternate wash formulation.	Be sure each unique wash solution is properly labeled. Prime the system thoroughly when switching between solutions.
Reader was malfunctioning or not blanked properly; this is a possible cause if the OD readings were high and the color was not dark.	Verify the reader's performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and reblank.
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.
Reagents were intermixed, contaminated or prepared incorrectly.	Ensure that the correct reagents were used, that working solutions were prepared correctly and that contamination has not occurred.