

NutriCulture LDH Cytotoxicity Assay Kit

Troubleshooting Guide

100 tests

Cat No: LCA100

Use this guide for common control, reaction-time, and absorbance problems with the NutriCulture LDH Cytotoxicity Assay Kit. The assay measures extracellular LDH released from damaged cells and uses high control, high blank, low control, and background blank wells. The datasheet specifies 3,000-10,000 cells per well, defined lysis and transfer volumes, light-protected reaction, stopping solution, and a 490 nm readout.

Before You Start - Quick Checks

1. Store the kit at -20°C protected from light.
2. Seed 3,000-10,000 cells per well in 100 uL medium.
3. Set up high control, high blank control, low control, and background blank exactly as described.
4. Avoid bubbles because they interfere with OD readings.
5. After adding Working Solution, protect the plate from light and determine an appropriate reaction time within 0-30 minutes.
6. After adding Stop Solution, measure absorbance immediately at 490 nm.

Problem	Most likely cause	Recommended action
High control and low control are too close	The maximum-release control may be insufficient or the assay window may not be well resolved.	Confirm that 10 uL Lysis Solution was added to the high control and high blank, and that the high-control minus low-control OD difference is greater than 0.2 as recommended.
Low control is unexpectedly high	Baseline LDH release from untreated cells may be high.	Use the low control to quantify spontaneous LDH release and subtract the background blank according to the datasheet calculation.
High blank is unexpectedly high	Background from medium plus Lysis Solution may be substantial.	Use the high blank control exactly as specified and subtract it from the high-control absorbance.
Sample background is high	The background blank may not have been included or subtracted.	Include medium-only background blank wells and subtract them from sample and low-control readings as defined in the data-processing section.
Sample OD exceeds the recommended linear range	The measured value may be outside the datasheet recommendation.	The datasheet recommends sample OD below 2.0 on the linear curve. Adjust the experimental setup so readings are evaluated in the recommended range.
OD varies strongly with read time	The reaction continues after Working Solution is added until it is stopped.	Determine the best reaction time for the cell type by checking 0, 5, 10, 20, and 30 minutes in the first experiment, as recommended.
Reaction is read too late without stopping	The assay may continue beyond the intended reaction interval.	Add 50 uL Stop Solution after the selected reaction time and immediately measure at 490 nm.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Bubbles create abnormal OD values	Air bubbles may be present in control or sample wells.	Avoid bubbles when adding Lysis Solution, transferring supernatant, and mixing Working Solution because the datasheet states bubbles interfere with OD reading.
Wrong volume is transferred to the detection plate	The supernatant transfer step may not match the protocol.	Transfer 50 uL supernatant from each sample/control well to a new 96-well plate, then add 50 uL Working Solution.
Controls were prepared in the wrong wells	The four-control design may have been confused.	Recreate the plate map: high control = cells + medium + Lysis Solution; high blank = medium + Lysis Solution; low control = cells + medium without lysis; background blank = medium only.
Signal is weak across all conditions	Cell number may be below the stated seeding range or reaction time may be too short.	Use 3,000-10,000 cells per well and establish an appropriate Working Solution reaction time within the 0-30 minute range.
Data calculation gives implausible cytotoxicity values	Background or control corrections may not match the datasheet formula.	Use Cytotoxicity (%) = $[(X-Z)/(Y-Z)] \times 100$ with X, Y, and Z defined from sample, high control, low control, and their corresponding blanks exactly as specified.
Working Solution was exposed to light during incubation	The protocol requires light protection during the color reaction.	Incubate the plate at room temperature protected from light, for example by wrapping it in aluminum foil.

Still having trouble? Recheck the four-control plate design, 3,000-10,000 cell seeding range, 10 uL lysis-control additions, 50 uL supernatant transfer, 50 uL Working Solution, light-protected 0-30 minute reaction, 50 uL Stop Solution, and immediate 490 nm reading. Confirm high-control minus low-control OD >0.2 and sample OD <2.0.