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Quantifying cell viability via LDH cytotoxicity assay

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes a procedure to assess the cytotoxicity of *in vitro* treatments by measuring the level of lactate dehydrogenase (LDH) in cell culture supernatants via an enzyme activity assay. LDH is a cytosolic enzyme released into the supernatant upon cell lysis. This protocol uses the Abcam LDH assay kit (ab102526), which measures the increase in NADH produced by the released LDH enzyme over time.

Materials

LDH Assay Kit (Cytotoxicity) **Abcam Catalog #**ab65393

Black 96-well microtiter plate
Plate reader
1.5 mL microcentrifuge tubes
15 mL centrifuge tubes
Cell culture supernatants (analyte)

Protocol materials

LDH Assay Kit (Cytotoxicity) **Abcam Catalog #**ab65393

Reagent preparation

10m

1 This protocol uses the LDH Assay Kit (Cytotoxicity) **Abcam Catalog #**ab65393. If you've already prepared kit reagents, skip to "Standard and substrate preparation."

2 Reconstitute NADH standard in 0.4 mL ultrapure water to generate a [M] 1.25 millimolar (mM) solution. Aliquot in 100 µL aliquots. Store remaining aliquots at -20 °C for future use.

3 Reconstitute recombinant LDH positive control in 200 µL LDH assay buffer. Aliquot in 20 µL aliquots. Store remaining aliquots at -20 °C for future use.

4 Reconstitute substrate mix in 1.1 mL ultrapure water. Allow to mix for ≥ 00:10:00, vortexing intermittently. Aliquot in 185 µL aliquots. Store remaining aliquots at -20 °C for future use. 10m

Standard and substrate preparation

5 Thaw LDH positive control on ice. Dilute entire aliquot to 200 µL in LDH assay buffer.

6 Thaw [M] 1.25 millimolar (mM) NADH standard aliquot on ice. Prepare standards as follows:

	A	B	C
	Volume NADH stock (µL)	**Volume assay buffer (µL)**	**[NADH] in well (µM)**
	0	125	0
	5	120	50
	10	115	100
	15	110	150

	A	B	C
	20	105	200
	25	100	250

7 Thaw LDH substrate aliquot on ice. Dilute 25× substrate to 1× in LDH assay buffer. Vortex to mix. Prepare ≥ 50 µL reaction mix per reaction (not including blank).

Sample preparation and analysis

32m

8 Analyze samples in technical triplicate. For each sample, prepare appropriate dilutions in LDH assay buffer in appropriate wells of a black, 96-well microtiter plate such that each well has a total volume of 50 µL. Depending on assay setup, you may need to optimize supernatant dilution factor.

Note

As an absorbance assay, the dynamic range of the LDH assay is limited. Ideally, the detected concentrations of NADH (which we use to calculate LDH activity) should fall within the range of the standards (50–250 µM). You may need to dilute analyte samples to achieve this.

In our hands, we found that no dilution was necessary for samples that didn't have significant supernatant LDH activity (i.e., when treatment had little impact on viability). For samples with significant LDH activity, a 5× dilution was generally appropriate. When testing unknown samples, we recommend analyzing both 1× and 5× dilutions.

9 Add standards (prepared in step 6) and controls to appropriate wells. Standards and controls are detailed in the following table:

	A	B	C
	Control	**Volume per well (µL)**	**Technical replicates**
	NADH standards	50	Duplicate
	LDH (1× dilution)	50	Triplicate

	A	B	C
	LDH (5× dilution)	10 (+ 40 µL buffer)	Triplicate
	Blank	100	Triplicate

10 Add 50 µL of reaction mix to all wells except the blank.

11 Measure absorbance at 450 nm on plate reader set to 37 °C in kinetic mode, taking readings every 00:00:00 for a total of 00:30:00 . 32m