ab65393 LDH-Cytotoxicity Assay Kit II

For the rapid, sensitive and accurate measurement of LDH-Cytotoxicity in cell culture samples.
This product is for research use only and is not intended for diagnostic use

Storage and Stability: All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied:

Item	Quantity	Storage Temperature (Before Prep)
Substrate Mix	1 vial	-20°C
LDH Assay Buffer	50 mL	-20°C
Lysis Buffer II	5 mL	-20°C or Ambient
Stop Solution IV	5 mL	-20°C
LDH Positive Control	1 vial	-20°C

Materials Required, Not Supplied

- These materials are not included in the kit, but will be required to successfully perform this assay:
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

Assay Protocol

1. Reconstitute LDH Positive Control with 100 µl of LDH Assay Buffer.

   Note: Aliquot LDH Positive Control after reconstitution. Store aliquots at -20 ˚C. Avoid freeze/thaw cycles.

2. **Sample Preparation:** Collect cells (adherent or suspension) and wash once with fresh regular culture medium, then seed 100 µl cells (with 2-10 x 10^4 cells*****) in a 96-well plate as the following:

   a. **Background Control:**

   100 µl culture medium per well in triplicates with no cells. The Background Control will measure reagents and LDH background from culture medium serum. The background value has to be subtracted from all other values.

   b. **Low Control:**

   100 µl cells in triplicate wells

   c. **High Control:**

   100 µl cells in triplicates, add 10 µl Lysis Buffer II each well, mix. To adjust the increase of medium volume, 11 µl of the medium may be used in LDH activity assay at step 5.

   d. **Test Sample:**

   100 µl cells in triplicates, add test substances each well, mix.

Notes:

a) Trypsin may be used to remove adherent cells from a culture surface before seeding in a 96-well plate.

b) The amount of cells to be used per well depends on the cell types. To optimize the assay, you can do a quick testing by using 2, 4, 8 x 10^4 cells per well, and then follow the assay protocol to determine the cell number you should use. The high control should be OD_{450nm} ~2.0 after 30 min treatment with 10 % Lysis Buffer II, while the low control should be OD_{450nm} < 0.8. The reaction time should be set at ~ 30 min.

c) LDH Positive Control (5 µl LDH) can be used to test whether all reagents are working properly to response to active LDH enzyme.

d) If the test substances are not dissolved in PBS, a solvent control may be performed by addition of the same amount of solvent in triplicates without testing substances.

3. **Sample Incubation:** Incubate cells in an incubator (5 % CO_2, 90 % humidity, 37°C) for the appropriate time of treatment determined for test substance. Gently shake the plate at end of the incubation to ensure LDH is evenly distributed in the culture medium.
4. Centrifuge cells at 600 x g for 10 min to precipitate the cells.
5. Transfer the clear medium solution (10 µl/well) into an optically clear 96-well plate.
6. **LDH Reaction Mix:** Reconstitute the Substrate Mix in 1.1 ml ddH_2O for 10 min and mix thoroughly. The solution is stable for two months at 4°C.

   For 100 assays, mix 200 µl of Substrate Mix with 10.0 ml of LDH Assay Buffer. The LDH Reaction Mix should be stable for several weeks at 4°C. Add 100 µl LDH Reaction Mix to each well, mix and incubate for 30 min****** at room temperature.
7. Measure the absorbance of all controls and samples with a plate reader equipped with 450 nm (440 - 490 nm) filter. The reference wavelength should be 650 nm.

Notes:

a) The reaction time can be decreased or increased depending on the color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be OD_{450nm} ~2.0, while the low control should be OD_{450nm} < 0.8.

b) The reaction can be stopped by adding 10 µl of Stop Solution IV, mix and read within 48 hours without significant changes. Protect the reaction from light and evaporation.

Data analysis

$$\text{Cytotoxicity (\%)} = \frac{\text{(Test Sample – Low Control)}}{\text{(High Control – Low Control)}} \times 100$$

Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the 10kDa spin column (ab93349)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Technical Support

For all technical or commercial enquiries please go to:

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