EZQuant™ Cytotoxicity Assay Kit

Cat# CA01, Size: 500 tests, Price $210
Cat# CA04, Size: 2000 tests, Price $475

Product Description

The EZQuant™ Cytotoxicity Assay Kit is used to assess cell death. It measures the level of LDH released when cells undergo stress or injury from chemicals that are toxic to the cells. The reagent is reduced by NADH that was oxidized by the extracellular LDH. Therefore, the amount of formazan produced is directly proportional to the number of dead cells.

Pair with EZQuant™ Cell Quantifying Kit

The EZQuant™ Cell Quantifying Kit conveniently determines the number of viable cells in cell proliferation and cytotoxicity assays. This product is useful for drug screening and cytotoxicity testing of chemicals. Meanwhile, the EZQuant™ Cytotoxicity Assay Kit determines the number of damaged cells and the degree of cytotoxicity. Combining these two products will allow researchers to fully understand the cell health conditions under various cytotoxic environments.

Features

- High linearity range
- Long shelf-life without degradation
  (2 months at 0-5° C)
- Simple homogenous assay or
  non-homogenous (multiplex) assay possible

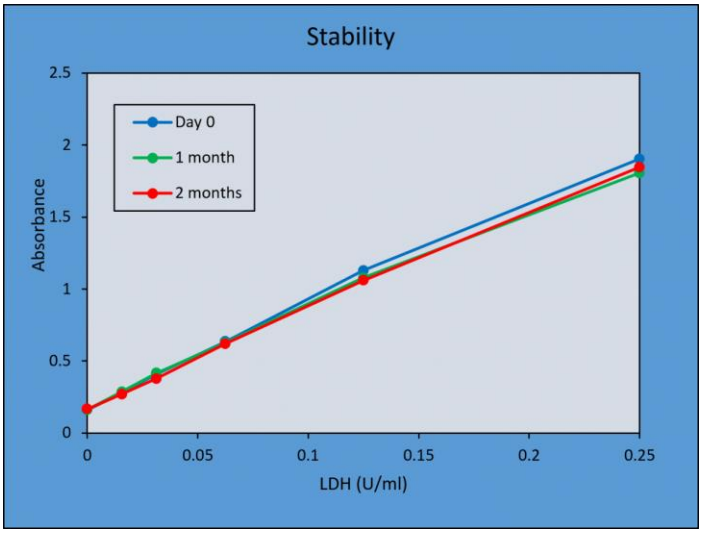

Figure 1. **High stability of EZQuant™ reconstituted working solution over a 2-month period.**

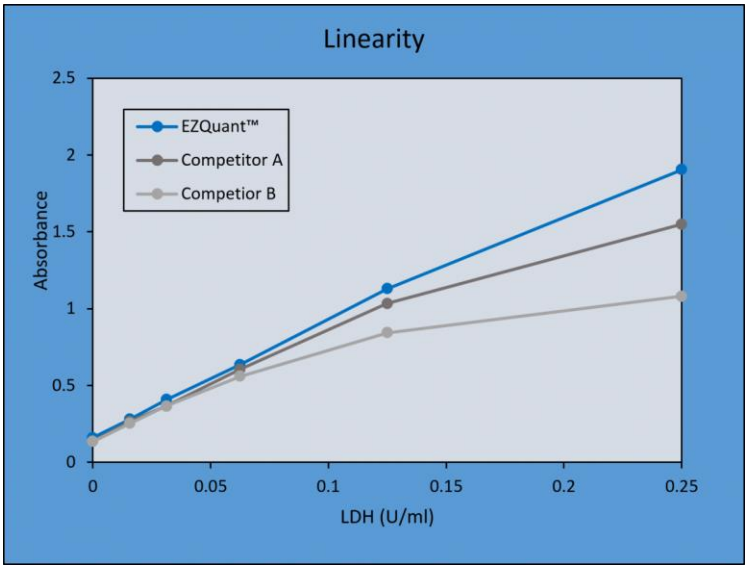

Figure 2. High linearity compared to other competitor products.

Product Specifications

Product Name	Cytotoxicity Assay Kit
Catalog #	CA01, CA04
Size	500 tests (CA01) / 2000 tests (CA04)
Shipping	Room Temperature
Storage and Stability	Store at 0-5° C
Quality Control	Appearance, blank, and sensitivity tests are performed for each lot. Only lots that pass the following criteria are offered: Blank Absorbance (460 nm) ≤ 0.700 Sensitivity Absorbance (460 nm) ≥ 1.000
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Protocol

Homogenous assay

Cell Concentration Optimization

1. Collect cells and wash them with the assay medium. Prepare cell suspension to 5×10^5 cells/ml in the assay medium.
2. Add 100 µl of the assay medium to each well of a flat-bottom 96-well tissue culture plate.
3. Prepare 2-fold serial dilution of each well in triplicate set of wells for the high-control, low-control and background control (medium only).
   Serial dilution: Add the cell suspension (5×10^5 cells/ml) to the first well and mix by pipetting. This well contains the maximum number of cells (2.5×10^4 cells/well). Transfer 100 µl from the first well to the next well, and mix by pipetting. Repeat this procedure.
4. Incubate the plate at 37° C for an appropriate time in a CO_2 incubator.
   Use the same incubation time in the cytotoxicity assay.
5. Add 10 µl of the lysis buffer to each well of the high control.
6. Incubate the plate at 37° C for 30 minutes in a CO_2 incubator.
7. Add 100 µl of the working solution to each well. Protect the plate from light and incubate it at the room temperature for 30 minutes.
8. Add 50 µl of the stop solution to each well.
9. Measure the absorbance at 490 nm by a microplate reader.

Cytotoxicity Assay

1. Add 50 µl of cell suspension to each well of a flat-bottom 96-well tissue culture plate.
   For adherent cells: incubate the plate at 37° C overnight in a CO_2 incubator to allow the cells to adhere and then replace the assay medium with 50 µl of fresh assay medium.
2. Add 50 µl of assay medium containing test substance that adjusted to the desired concentration.

	Background control	Test substance	Low control	High control
Assay medium	100 µl	N/A	50 µl	50 µl
Cell suspension	N/A	50 µl	50 µl	50 µl
Test substance in culture medium	N/A	50 µl	N/A	N/A
Lysis buffer	N/A	N/A	N/A	10 µl

3. Incubate the plate at 37° C for an appropriate time period in a CO_2 incubator.
4. Add 10 µl of the lysis buffer to each well of the high control. Incubate the plate at 37° C for 30 minutes in a CO_2 incubator.
5. Add 100 µl of the working solution to each well. Protect the plate from light and incubate it at the room temperature for 30 minutes.
6. Add 50 µl of the stop solution to each well.
7. Measure the absorbance at 490 nm by a microplate reader.

Calculate Cytotoxicity

Calculate the average absorbance from each triplicate set of wells and subtract the background control value from each absorbance one. Calculate the percent cytotoxicity by the following equation:
Cytotoxicity (%) = (Test substance - Low control) / (High control - Low control) x 100

Non-homogenous assay

Cell Concentration Optimization

1. Collect cells and wash them with the assay medium. Prepare cell suspension to 5×10^5 cells/ml in the assay medium.
2. Add 100 µl of the assay medium to each well of a 96-well tissue culture plate.
   Use round or v-bottomed plate for suspension cells, flat-bottomed plate for adherent cells.
3. Prepare 2-fold serial dilution of each well in triplicate set of wells for the high-control, low-control and background control (medium only).
   Serial dilution: Add the cell suspension (5×10^5 cells/ml) to the first well and mix by pipetting. This well contains the maximum number of cells (2.5×10^4 cells/well). Transfer 100 µl from the first well to the next well, and mix by pipetting. Repeat this procedure.
4. Add 100 µl of the assay medium to each well.
5. Incubate the plate at 37° C for an appropriate time in a CO_2 incubator.
   Use the same incubation time in the cytotoxicity assay.
6. Add 20 µl of the lysis buffer to each well of the high control.
7. Incubate the plate at 37° C for 30 minutes in a CO_2 incubator.
8. Centrifuge the plate at 250 × *g* for 2 minutes to precipitate the cells (for suspension cells).
9. Transfer 100 µl of the supernatant from each well to an optically clear 96-well plate.
10. Add 100 µl of the working solution to each well. Protect the plate from light and incubate it at the room temperature for 30 minutes.
11. Add 50 µl of the stop solution to each well.
12. Measure the absorbance at 490 nm by a microplate reader.

Cytotoxicity Assay

1. Add 100 µl of the cell suspension to each well of a 96-well tissue culture plate.
   For adherent cells: incubate the plate at 37° C overnight in a CO_2 incubator to allow the cells to adhere and then replace the assay medium with 100 µl of fresh assay medium.
2. Add 100 µl of assay medium containing test substance that adjusted to the desired concentration.

	Background control	Test substance	Low control	High control
Assay medium	220 µl	20 µl	120 µl	100 µl
Cell suspension	N/A	100 µl	100 µl	100 µl
Test substance in culture medium	N/A	100 µl	N/A	N/A
Lysis buffer	N/A	N/A	N/A	20 µl

3. Incubate the plate at 37° C for an appropriate time period in a CO_2 incubator.
4. Add 20 µl of the lysis buffer to each well of the high control. Incubate the plate at 37° C for 30 minutes in a CO_2 incubator.
5. Centrifuge the plate at 250 × *g* for 2 minutes to precipitate the cells (for suspension cells).
6. Transfer 100 µl of the supernatant from each well to each well of a new optically clear 96-well plate.
7. Add 100 µl of the working solution to each well. Protect the plate from light and incubate it at room temperature for 30 minutes.
8. Add 50 µl of the stop solution to each well.
9. Measure the absorbance at 490 nm by a microplate reader.

Calculate Cytotoxicity

Calculate the average absorbance from each triplicate set of wells and subtract the background control value from each absorbance one. Calculate the percent cytotoxicity by the following equation:
Cytotoxicity (%) = (Test substance - Low control) / (High control - Low control) x 100

Publications:

Peters, Molly C., et al. **"A Novel Polyamine-Targeted Therapy for BRAF Mutant Melanoma Tumors."** *Medical Sciences* 6.1 (2018): 3.