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A Geno Technology, Inc. (USA) brand name

Cytoscan™-LDH Cytotoxicity Assay

A Colorimetric Assay for Cellular Cytotoxicity

(Cat. # 786-210, 786-324)



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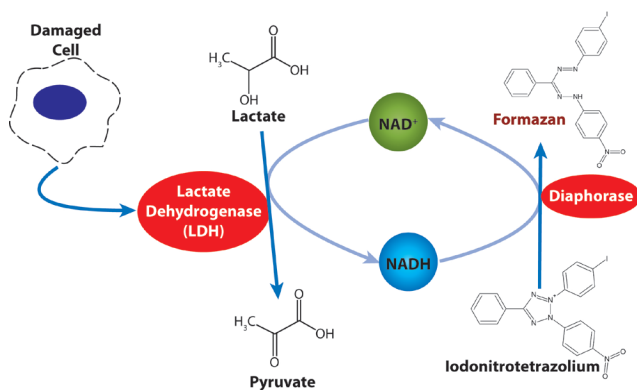
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INTRODUCTION

The G-Biosciences' Cytoscan™ LDH Cytotoxicity Assay is a simple, reliable colorimetric method of quantitatively assaying cellular cytotoxicity. The assay can be used with different cell types for assaying cell mediated cytotoxicity as well as cytotoxicity mediated by chemicals and other test compounds.

An alternative to ⁵¹Cr release cytotoxicity assays; the kit combines the advantages of reliability and simple evaluation, characteristic of radioisotope release assays, with the convenience of accuracy and avoidance of radioactivity.

The assay quantitatively measures a stable cytosolic enzyme lactate dehydrogenase (LDH), which is released upon cell lysis. The released LDH is measured with a coupled enzymatic reaction that results in the conversion of a tetrazolium salt (INT) into a red color formazan. The LDH activity is determined as NADH oxidation or INT reduction over a defined time period (see below). The enzymatic reactions associated with the assay are as follows:



The resulting formazan absorbs maximally at 492nm and can be measured quantitatively at 490nm using a micro-plate reader or spectrophotometer.

ITEM(S) SUPPLIED

Description	Cat. # 786-210 1000 assays	Cat. # 786-324 200 assays
Cytoscan™ Substrate Mix (lyophilizate)	5	1
Cytoscan™-LDH Assay Buffer	5 x 0.6ml	1 x 0.6ml
Cytoscan™-LDH Lysis Buffer [10X]	12ml	2.5ml
Cytoscan™-LDH Stop Solution	60ml	12ml
LDH Positive Control	30µl	6µl

STORAGE CONDITION

The kit is shipped at ambient temperature. Store Cytoscan™ Substrate Mix and Assay Buffer at -20°C. LDH Positive Control, 10X Lysis buffer and Stop Solution can be stored at 4°C. The kit is stable for 12 months, when stored as recommended.

ADDITIONAL ITEMS REQUIRED

- Microplate reader
- 96 well tissue culture plate
- Multichannel pipette
- PBS containing 1% BSA (bovine serum albumin) solution for LDH Positive Control

PROCEDURE SUMMARY

Cultured cells are incubated with chemical compounds (e.g., Actinomycin D) or effector cells (e.g., natural killer cells) to induce cytotoxicity and subsequently release LDH. The LDH released into the medium is transferred to a new plate and mixed with Reaction Mixture. After a 30 minute room temperature incubation, reactions are stopped by adding Stop Solution. Absorbance at 490nm and 680nm is measured using a plate-reading spectrophotometer to determine LDH activity.

IMPORTANT INFORMATION

- LDH activity varies in different sera (e.g., horse serum, fetal bovine serum and calf serum) commonly used to maintain different types of mammalian cell lines. Therefore, it is important to measure LDH activity in culture media with serum. The inherent LDH activity present in serum causes background signal in the assay. To reduce background signal, use the minimum serum percentage appropriate for each cell line; however, ensure the minimum serum percentage does not affect cell viability for the assay period.
- All procedures are written for 96-well plates; for 384-well plates, reduce all volumes by four-fold

REAGENT PREPARATION

Reaction Mixture

1. Dissolve one vial of the provided Substrate Mix in total volume of 11.4ml ultrapure water in a 15ml conical tube. Mix gently to fully dissolve the lyophilizate.
2. Thaw one vial of Assay Buffer (0.6ml) to room temperature
NOTE: *Protect Assay Buffer from light and do not leave at room temperature for longer than necessary.*
3. Prepare Reaction Mix by combining 0.6ml Assay Buffer with 11.4ml Substrate Mix in a 15ml conical tube. Mix by inverting gently and protect from light until use.
NOTE: *One vial of Reaction Mixture is enough for two 96-well plates. The remaining Reaction Mixture can be stored at -20°C protected from light for 3-4*

weeks with tolerance for three freeze/thaw cycles without affecting the activity within the storage period.

1X LDH Positive Control

1. Dilute 1 μ l LDH Positive Control with 10ml 1% BSA in PBS.

PROTOCOL

The Cytoscan™ LDH Assay protocol is divided into three sections – Determination of Optimum Cell Number for LDH Cytotoxicity Assay, Cell-Mediated Cytotoxicity Assay and Chemical Induced Cytotoxicity Assay. The various steps of the protocol are as follows:

(A) DETERMINATION OF OPTIMUM CELL NUMBER FOR LDH CYTOTOXICITY ASSAY

Different cell types have different levels of LDH activity. For optimal results, it is highly recommended to do a preliminary experiment using your target cell population(s) to determine the optimum number of target cells to be used with the Cytoscan™ LDH Assay to ensure LDH signal is within the linear range. The supplied LDH may be used as a positive control to verify that LDH assay is functioning properly.

I. Assay Plate Setup

1. First prepare serial dilutions of target cell type (0, 5000, 10,000 and 20,000/100 μ l media) in two sets of triplicate wells in a 96-well tissue culture plate.
NOTE: Use the same medium and final volume that will be used for cytotoxicity assays. For example, if the co-culture volume is 50 μ l/well of target cells with 50 μ l/well of effector cells, prepare serial dilutions in 100 μ l/well.
2. Prepare a triplicate set of wells for a complete medium control without cells to determine LDH background activity present in sera used for media supplementation and a triplicate set of wells for a serum-free media control without cells to determine the amount of LDH activity in sera.
3. Incubate the plate at 37°C, 5% CO₂ overnight.
4. **Optional LDH Assay:** To perform a LDH positive control assay, mix the supplied LDH Positive Control properly and then dilute it 1:10,000 (1 μ l of LDH into 10ml of PBS containing 1% BSA). For LDH positive control assay, use the same assay volume (100 μ l) as in the wells containing cells in triplicate.

II. Cell Lysis and Harvesting Supernatant

1. Add 10 μ l of sterile, ultrapure water to one set of triplicate wells containing cells.
NOTE: These cells are referred to as Spontaneous LDH Activity Controls.
2. Add 10 μ l of Lysis Buffer [10X] to the other set of triplicate cell-containing wells and mix by gentle tapping.
NOTE: These cells are referred to as Maximum LDH Activity Controls.
3. Incubate in a humidified chamber at 37°C, 5% CO₂ for 45 minutes.

III. LDH Measurement

1. Transfer 50µl of each sample medium (i.e. serum-free medium, complete medium, Spontaneous LDH Activity Controls, Maximum LDH Activity Controls and 1X LDH Positive Control) to a 96 well flat bottom plate in triplicate wells.

NOTE: To perform a LDH positive control assay, use 50µl 1X LDH Positive Control in triplicate wells..

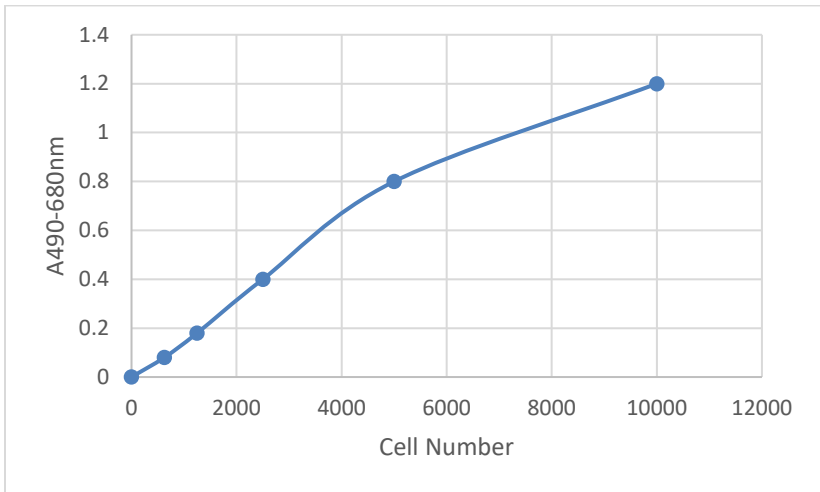
2. Add 50µl Reaction Mixture to each well and mix by gentle tapping.
3. Cover the plate with foil to protect it from light and incubate at room temperature for 30 minutes or at 37°C for 20 minutes.

4. Add 50µl of Stop Solution to each well and mix with gentle tapping.

NOTE: Break any bubbles present in wells with a pipette tip, syringe needle or centrifugation before reading.

5. Measure the absorbance at 490nm and 680nm. To determine the LDH activity, subtract 680nm absorbance value (background signal from instrument) from the 490nm absorbance.

6. Plot the Maximum LDH Release Control absorbance value minus the Spontaneous LDH Release Control absorbance value versus cell number to determine the linear range of the LDH cytotoxicity assay and the optimal number of cells.



Graph of CHO-K1 cells LDH Activity vs. cell number.

(B) CELL-MEDIATED CYTOTOXICITY ASSAY

1. Perform each control and experimental assay in triplicate and prepare the 96-well assay plate as follows:
 - a. **Experimental Wells:** Add a constant number of target cells (e.g. Jurkat, K562, etc.), as optimized previously, to all experimental wells. Add various numbers of effector cells (e.g. natural killer cells, lymphokine-activate cells, cytotoxic T lymphocytes or other cell lines) to triplicate set of wells to test several effector:target cell ratios. The final combined volume should be 100µl/well.
 - b. **Effector Cell Spontaneous LDH Release Control:** This control corrects for spontaneous release of LDH from effector cells. Add effector cells at each concentration used in the experimental setup to a triplicate set of wells containing medium. Adjust the final volume to 100µl/well with culture medium.
 - c. **Target Cell Spontaneous LDH Release Control:** This control corrects for spontaneous release from target cells. Add target cells (as optimized previously) to a triplicate set of wells containing culture medium. Adjust the final volume to 100µl/well with culture medium.
 - d. **Target Cell Maximum LDH Release Control:** This control is required in calculations to determine 100% release of LDH. Add the same number of target cells used in the Experimental Wells. The final volume must be 100µl/well.
 - e. **Volume Correction Control:** This control is recommended for correcting the volume increase caused by addition of 10X Lysis Buffer. This volume change affects the concentration of serum, which contribute to the absorbance values. Add 100µl culture media to a triplicate set of wells.
 - f. **Culture Medium Background Control:** This control is required to correct for the contributions caused by LDH activity that may be present in serum containing culture medium. Add 100µl of culture medium to a triplicate set of wells (without cells).
2. Add 10µl sterile, ultrapure water to one set of triplicate wells containing Effector and Target Cell Spontaneous LDH Release Controls.
3. Incubate the plate in an incubator at 37°C, 5% CO₂ for an appropriate time.
NOTE: *A minimum of 4 hours incubation is needed for sufficient contact between target and effector cells.*
4. 45 minutes before harvesting the supernatant, add 10µl of 10X Lysis Buffer to the wells containing the Target Cell maximum LDH Release Control and Volume Correction Control.
5. At the end of incubation, centrifuge the plate at 250x g for 3 minutes.
6. Transfer 50µl of each sample to a 96-well flat-bottom plate in triplicate wells by using a multichannel pipette.
7. Add 50µl Reaction Mix to each well of the plate and mix by gently tapping.
8. Incubate at room temperature for 30 minutes protected from light.

9. Add 50µl Stop Solution to each sample and mix by gentle tapping.
10. Measure the absorbance at 490nm and 680nm. To determine the LDH activity, subtract 680nm absorbance value (background signal from instrument) from the 490nm absorbance before calculating the % Cytotoxicity.
NOTE: Break any bubbles present in wells with a pipette tip, syringe needle or centrifugation before reading.
11. To calculate the corrected values, subtract the average value of the culture medium background control from average values of the Experimental, Effector Cell Spontaneous LDH Release Control and Target Cell Spontaneous LDH Release Control. The average value of the Volume Correction Control is then subtracted from the average value of the Target Cell Maximum LDH Release Control.
12. To calculate % Cytotoxicity for each Effector:Target cell ratio, use the equation below with the corrected values:

$$\frac{\text{Experimental value} - \text{Effector Cells Spontaneous Control} - \text{Target Cells Spontaneous Control}}{\text{Target Cell Maximum Control} - \text{Target Cells Spontaneous Control}} \times 100$$

(C) CHEMICAL COMPOUND INDUCED CYTOTOXICITY ASSAY:

The Cytoscan™ LDH Assay can also be used to test for high-throughput screening of cell death of a single cell type (without an effector cell) due to cytotoxicity of the test compounds.

1. Place the optimal number of cells/well in 100µl of medium, as determined previously, in triplicate wells in a 96-well tissue culture plate.
2. **Controls:** Include the following controls on the 96-well plate:
 - a. A complete media control without cells to determine LDH background activity present in the sera used.
 - b. A serum-free media control to determine the amount of LDH activity in the sera.
 - c. Spontaneous LDH Activity Controls: Plate additional cells in triplicate wells.
 - d. Maximum LDH Activity Controls: Plate additional cells in triplicate wells.
3. Incubate the plate in an incubator at 37°C, 5% CO₂ overnight.
4. Prepare the wells as follows:
 - a. Spontaneous LDH Activity Controls: Add 10µl sterile, ultrapure water to one set of triplicate wells.
 - b. Maximum LDH Activity Controls: Add nothing to one set of triplicate wells of cells.
 - c. Chemical Compound: Add 10µl of vehicle containing chemical compound to one set of triplicate wells of cells.
5. Incubate the plate in an incubator at 37°C, 5% CO₂ as needed.

6. To the Maximum LDH Activity Control wells, add 10µl Lysis Buffer [10X] and mix by gently tapping.
7. Incubate the plate in an incubator at 37°C, 5% CO₂ for 45 minutes.
8. Transfer 50µl each sample medium to a 96-well flat-bottomed plate in triplicate wells. Samples should include:
 - a. Complete Medium
 - b. Serum-free medium
 - c. Spontaneous LDH Activity Controls
 - d. Compound-treated Samples
 - e. Maximum LDH Activity Controls
9. Add 50µl Reaction Mixture to each sample and mix with a multichannel pipette.
10. Incubate at room temperature for 30 minutes protected from light.
11. Add 50µl Stop Solution to each sample and mix by gentle tapping.
NOTE: Break any bubbles present in wells with a pipette tip, syringe needle or centrifugation before reading.
12. Measure the absorbance at 490nm and 680nm. To determine the LDH activity, subtract 680nm absorbance value (background signal from instrument) from the 490nm absorbance before calculating the % Cytotoxicity.

$$\% \text{ Cytotoxicity} = \frac{\text{Compound Treated} - \text{Spontaneous LDH Activity}}{\text{Maximum LDH release} - \text{Spontaneous LDH Activity}} \times 100$$

TROUBLESHOOTING

Problem	Possible Cause	Solution
High medium control absorbance	High inherent LDH activity in animal serum in cell culture media	Reduce serum concentration to 1-5%
High spontaneous control absorbance	High cell density	Repeat determination of optimum cell number for assay
	Vigorous pipetting during cell plating	Gently handle cell suspension during plate set-up
Low absorbance value in experiment	Cell density too low	Repeat determination of optimum cell number
High variability of absorbance well-to-well	Bubbles were in well	Centrifuge the plate for a longer time or at a higher speed
		Break bubbles with a syringe needle

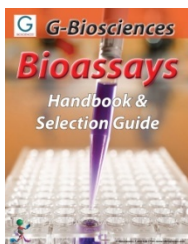
CITATIONS

1. Botta, A. et al (2019) *Nutrition & Metabolism*. 16:14
2. Al-Fatlawi, A. A. Y (2019) *Biosci Res* .16:695.
3. Hsu, H. T. et al (2018) *BMC Complementary and Alternative Medicine*. 18:211
4. Wang, J. et al (2018) *Biomed. Pharmacother.* 108:707
5. Tseng, Y.T. et al (2017) *Phytomedicine*. 34:97
6. Ahmad, I. et al (2017) *J Plant Biochem Physiol*.5:1
7. Stephan, H. et al (2017) *Chem Plus Chem*. 82:638
8. Levy J.M.M., et al (2017) *Elife*. 6:e19671
9. Ailte, I. et al (2017) *Traffic*. 18:176
10. Liu, Y.H. et al (2016) *J Prob Health*. 4:2.
11. Balkhi, M. et al (2016) *Curr Drug Targets CNS Neurol Disord* 15:54
12. Prashanthi, K. at al (2015) *Braz. arch. biol. technol*.58:6
13. Thomas, M. G. et al (2015) *Toxicol In Vitro*. doi:10.1016/j.tiv.2015.10.007
14. Bose, C. et al (2015) *PLoS ONE* 10(8): e0136563
15. Quave, C.L. et al (2015) *PLoS ONE* 10(8): e0136486
16. Blackler, R.W. et al (2015) *Am J Physiol*. DOI: 10.1152/ajpgi.00066.2015
17. Benien, P. et al (2015) *J. Liposome. Res*. DOI: 10.3109/08982104.2015.1022557
18. Li, W. H. et al (2015) *Dermatol Ther*. 5:53
19. Cao, S. et al (2014) *BJP*. 172:1869
20. Dedinszki, D. et al (2014) *Cellular Signalling*. 27:363
21. Al-Fatlawi, A.A. et al (2014) *Pharm Biol*. doi:10.3109/13880209.2014.911919
22. Al-Fatlawi, A.A. et al. (2014) *World J Pharm Sci* 2: 1218
23. Mulcahy, J. M. et al (2014) *Cancer Discovery* 4:773
24. Wang, Q. et al (2014) *Transl. Stroke Res*. 10.1007/s12975-014-0342-1
25. Kulkarni, P. S. et al (2014) *Mol. Pharmaceutics*. 11:2390
26. Al-Ftlawi, A. et al (2014) *Int J Pharm Pharm Sci*. 6:515
27. Maycotte, P. et al (2014) *Cancer Res*. 74:2579
28. Hsu, Y. et al (2014) *Neurotox. Res*. 25:262
29. Vong, L. et al (2014) *J. Immunol*. doi: 10.4049/jimmunol.1302286
30. Park, G. et al (2014) *Skin Pharmacol. Physiol*. 27:132
31. Chen, T. et al (2014) *Exp. Neurol*. 255:127
32. Connell, B.J. et al (2014) *Neurosci. Letters*. 561:151
33. Ossa, J.C. et al (2013) *Cell. Microbiol*. 15:446
34. Leonardo, C.C. et al (2013) *Eur. J. Neurosci*. 38:3659
35. Yang, C. et al (2013) *ACS Appl. Mater. Interfaces*. 5:10985
36. Swaminathan, S. et al (2013) *Am. J. Pathol*. 183:796
37. Hsu, Y. et al (2013) *Toxic. Appl. Pharmacol*. 272:787
38. Li, W. et al (2013) *J. Dermat. Sci*. 71:58
39. Ling, J. et al (2013) *BBA-Mol. Cell. Biol. L*. 1831:387
40. Ho, Nathan K. et al (2012) *Infect. Immun*. 80:2307

41. Lo, Y. et al (2012) Evid-Based Compl Alt.
<http://dx.doi.org/10.1155/2012/501032>
42. Hsu, Y. et al (2012) Eur. J. Pharma. Sci. 46:415
43. Westwell-Roper, C. et al (2011) J Immunol 187:2755
44. Tomassian, T. et al (2011) J Immunol 187:2993
45. Sharma, S. et al (2011) Int J Toxicology. 30:358
46. Fan, C. et al (2010) Toxicol Appl. Pharmacol. 245:1
47. Shih, Y. et al (2010) Free Radical Bio. Med. 49:1471
48. Agashe, H. et al (2010) Colloid Surface B. 75:573
49. Tarnawski, A. (2005) Biochem. Biophys. Res. Comm. 334:207
50. Round, J.L. et al (2005) J Exp Med 201:419
51. Chen, A. and Xu, J. (2005) Am J Physiol Gastr Liver Physiol 288:G447
52. Bose, C. et al (2005) Am J Physiol Gastr Liver Physiol 289:G926
53. Kodavanti, P.R.S. and Ward, T.R (2005) Toxicol. Sci 85:952
54. Haslam, G. et al (2005) Anal. Biochem. 336:187
55. Chiu, S. et al (2005) Gastroenterology. 128:63

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