Lonza
www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # Inst-LT07-217 07/11
© 2011 Lonza Walkersville, Inc.

ToxiLight™ bioassay kit

Non destructive cytotoxicity assay

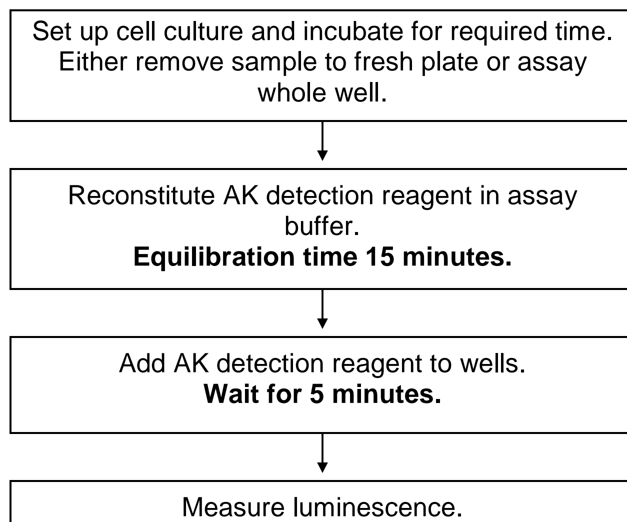
Safety

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Instructions for use

ToxiLight™ assay procedure

(For detailed assay protocol see specific protocol pg.3)



Kit contents

LT07-217 500 tests (Sufficient for 5 plates)
1. LT27-060 AK detection reagent. Lyophilized 5 x 10 ml vials.
2. LT27-066 AK assay buffer. 1 x 50 ml bottle.

LT17-217 500 tests (Sufficient for 5 plates)
1. LT27-060 AK detection reagent. Lyophilized 5 x 10 ml vials.
2. LT27-066 AK assay buffer. 1 x 50 ml bottle.
3. 5 x 96 well white walled microplates.

LT07-117 1000 tests (Sufficient for 10 plates)
1. LT27-061 AK detection reagent. Lyophilized 5 x 20 ml vials.
2. LT27-066 AK assay buffer. 2 x 50 ml bottles.

The kit should be stored at 2°C-8°C. Do not freeze. See kit label for expiry date of the whole kit. See bottle labels for expiry dates of individual components.

Available separately

LT07-517 ToxiLight™ 100% lysis reagent set. (Sufficient for 200 tests).

Intended use

The ToxiLight™ bioassay is a non-destructive bioluminescent cytotoxicity assay designed to measure toxicity in mammalian cells and cell lines in culture. The kit quantitatively measures the release of adenylate kinase (AK) from damaged cells. It is a safe, convenient, and highly sensitive method for measuring cytolysis. The assay can be conducted on aliquots of culture media, leaving the remaining cell culture for further testing; for example reporter gene assays. Alternatively, the assay can be performed directly on cells cultured in a microtitre plate.

The assay has been designed for use in 96 or 384 well microtitre plates. A full plate can be processed in less than ten minutes. If desired, the assay can also be performed in larger volumes using cuvettes.

Principles

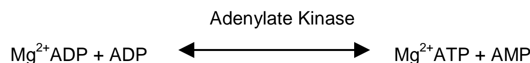
The kit is based on the bioluminescent measurement of AK which is present in all cells. A loss of cell integrity, through damage to the plasma membrane, results in the leakage of a number of factors from cells cultured in vitro into the surrounding medium. The measurement of the release of AK from the cells allows the accurate and sensitive determination of cytotoxicity and cytolysis.

The reaction involves two steps. The first involves the addition of ADP as a substrate for AK. In the presence of the enzyme, AK, the ADP is converted

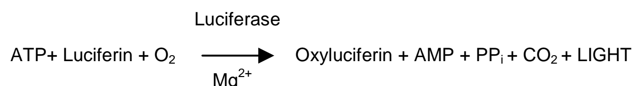
All trademarks herein are marks of Lonza Group or its subsidiaries.
Developed by Lonza Nottingham, Ltd.

Lonza

to ATP for assay by bioluminescence:



The bioluminescent method utilizes an enzyme luciferase, which catalyses the formation of light from ATP and luciferin according to the following reaction:



By combining the two reactions, the emitted light intensity is linearly related to the AK concentration and is measured using a luminometer or beta counter. The ToxiLight™ bioassay offers many advantages over conventional methods for measuring cytotoxicity, as there is no requirement for the use of radioisotopes or detergents. In addition, it only requires minimal amounts of culture media/cells, allowing the remainder to be used for other experiments.

Outline of the method

- The kit contains all the required reagents to perform the assay.
- For additional equipment required to perform the assay please see the equipment section.
- Maximum seeding density. (See appendix).
  96 well plate - 10,000 adherent or 50,000 suspension cells/well
  384 well plate - 2,500 adherent or 12,500 suspension cells/well
- Recommended culture volume
  96 well plate - 100 µl
  384 well plate - 25 µl
- Add AK detection reagent.
- Wait 5 minutes for signal generation.
- Read luminescence.

Selection of protocol

In order to select the correct protocol for your assay please determine the answers to the following questions:

1. Is the plate size 96 or 384 wells?
2. Are the plates used for culture compatible with luminescence detection (usually opaque, white or black walled with clear bottoms)?
3. Will the culture be required for another assay i.e. will sampling / transfer of supernatant be needed.

The table below can then be used to select the most suitable protocol:

Protocol	Plate size	Culture plates-luminescence compatible?	Sampling
1	96 well	Y	N
2	96 well	N	Y
3	384 well	Y	N
4	384 well	N	Y

For cells grown in a luminescence incompatible plate please follow the protocol for supernatant samples.

Reagent reconstitution and storage

Please read this section carefully to ensure optimal performance for your assay. This procedure requires at least 15 minutes equilibration time.

1. AK detection reagent (AKDR)

- Add 10 ml (5 plate kit) or 20 ml (10 plate kit) of assay buffer to the vial containing the lyophilized AK detection reagent.
- Replace the blue screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

Use reconstituted reagent within 6 hours, or 24 hours if stored at 2°C-8°C. Unused reagent can be aliquotted into polypropylene tubes and stored at –20°C for up to two months. Once thawed, reagent must not be refrozen and the reagents should be allowed to reach room temperature before use, without the aid of artificial heat.

2. Assay buffer

This is provided ready for use. Store at 2°C-8°C when not in use.

Equipment

1. Instrumentation

The ToxiLight™ kit requires the use of a luminometer or beta counter. The parameters of the luminometer/beta counter should be assessed and the conditions below used to produce the correct programming of the machine. If the luminometer has temperature control this should be set to 22˚C, the optimal temperature for luciferase.

All trademarks herein are marks of Lonza Group or its subsidiaries.
Developed by Lonza Nottingham, Ltd.

Microplate luminometers

- Read time 1 second (integrated)

Cuvette/tube luminometers:

- Read time 1 second (integrated)

Beta counters

- Mode - out of coincidence or luminescence
- Read time 1 second (integrated)

The assay requires a delay time of 5 minutes to ensure signal generation. An immediate reading may not give the reagent time to detect all the AK present in the culture.

2. Additional equipment and consumables

a) 10 ml sterile pipettes.
b) Either clear bottomed, white walled tissue culture treated plates* for combined culture and measurement.
c) Or opaque white microtitre plates suitable for luminescence measurements.
The same microplates should be used with beta counters.
d) Multichannel micropipettes - 50-200 µl and 5-50 µl

** The clear bottomed, white walled tissue culture treated plates for combined culture and measurement are supplied as part of a 5 plate ToxiLight™ kit by Lonza (product code LT17-217) or as a separate product (product code LT07-102; box of 25 plates).*

Selection of protocols

To ensure that the optimal performance of the assay is achieved for your experiment please make certain that you have carefully read the reagent reconstitution and storage procedure and also have fully reviewed the checklist on pg 2 for the correct protocol selection.

Please note that protocols 2 and 4 include an extra transfer step.

Protocol 1: Adherent/suspension cells
Cells cultured in luminescence compatible plate; 96 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AK detection reagent (AKDR) in assay buffer (see pg 2). Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Remove the culture plate from the incubator and allow it to cool to room temperature for at least 5 minutes.
4. Program the luminometer to take an immediate 1 second integrated reading of appropriate wells.
5. Add 100 µl of AKDR to each well and wait 5 minutes.
6. Place plate in luminometer and initiate the program.

Protocol 2: Adherent/suspension cells
Supernatant sampling procedure
Cells cultured in luminescence incompatible plate; 96 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AK detection reagent (AKDR) in assay buffer (see pg 2).
3. Leave for 15 minutes at room temperature to ensure complete rehydration.
4. Remove the culture plate from the incubator and allow it to cool to room temperature for at least 5 minutes.
5. Program the luminometer to take an immediate 1 second integrated reading of appropriate wells.
6. Transfer 20 µl of cell supernatant to a luminescence compatible 96 well plate.
7. Add 100 µl of AKDR to each well and wait 5 minutes.
8. Place plate in luminometer and initiate the program.

Protocol 3: Adherent/suspension cells
Cells cultured in luminescence compatible plate; 384 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AK detection reagent (AKDR) in assay buffer (see pg 2). Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Remove the culture plate from the incubator and allow it to cool to room temperature for at least 5 minutes.
4. Program the luminometer to take an immediate 1 second integrated reading of appropriate wells.
5. Add 25 µl of AKDR to each well and wait 5 minutes.
6. Place plate in luminometer and initiate the program.

Protocol 4: Adherent/suspension cells
Supernatant sampling procedure
Cells cultured in luminescence incompatible plate; 384 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AK detection reagent (AKDR) in assay buffer (see pg 2). Leave for 15 minutes

All trademarks herein are marks of Lonza Group or its subsidiaries.
Developed by Lonza Nottingham, Ltd.

at room temperature to ensure complete rehydration.
3. Remove the culture plate from the incubator and allow it to cool to room temperature for at least 5 minutes.
4. Program the luminometer to take an immediate 1 second integrated reading of appropriate wells.
5. Transfer 5 µl of cell supernatant to a luminescence compatible 384 well plate.
6. Add 25 µl of AKDR to each well and wait 5 minutes.
7. Place plate in luminometer and initiate the program.

Interpretation of results

In bioluminescent cytotoxicity assays the direct luminometer light output (commonly RLUs) or beta counter light output (cpm) may be used to calculate the cell responses (directly analogous to using cpm in radioisotope based assays).

Adenylate kinase will only leak from cells whose cell integrity has been compromised. Once the plasma membrane has been damaged by cytotoxic action the AK will be available for detection in the surrounding medium. Cells that have not progressed to this point in the cell death pathway will not be detected by the ToxiLight™ assay. It is possible to achieve a total adenylate kinase control by using the ToxiLight™ 100% lysis reagent. This kit is available separately (LT07-517) and is intended for use with ToxiLight™ non-destructive cytotoxicity assay.

References

Crouch, S., Kozlowski, R., Slater, K., Fletcher, J.(1993) The use of ATP Bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods* **160(1)**:81-8

Olsson, T. et al. (1983) Leakage of adenylate kinase from stored blood cells. *J. Appl. Biochem* **5**, 347-445.

Squirrell, D. and Murphy, J.(1997) Rapid detection of very low numbers of micro-organisms using adenylate kinase as a cell marker. *A practical guide to industrial uses of ATP luminescence in rapid microbiology* p.107-113.

Appendix

Seeding density

It is suggested that adherent cells are seeded at no more than 10,000 and 2,500 cells/well in 96 and 384 wells respectively. For suspension cells this can be increased to 50,000 and 12,500 cells/well in the respective formats.

If the experimental protocol requires much higher cell numbers, then a reduction in the amount of adenylate kinase supplied to the assay is required. This can be achieved by reducing the sample volume of the supernatant from the cultured wells.

Troubleshooting

High background levels?

AK: Fetal calf serum does contain levels of AK which can differ between manufacturers and between batches of sera. The ToxiLight™ bioassay has been optimized to perform in the presence of serum contaminating AK but it is recommended that an assessment of the levels of AK in sera is included in batch testing if possible.

ATP: Take great care when handling any of the reagents. Skin has high levels of ATP on its surface that can contaminate the reagents leading to falsely high readings. Wear latex gloves or the equivalent.

At all times the luminometer dispensing lines must be kept scrupulously clean. This is of particular importance when luminometers are also being used for luciferase reporter gene assays. Any residual enzyme in the dispensing lines must be removed using a cleaning reagent that neutralizes the enzyme. ExPro™ cleaning solution is supplied by Lonza as a separate product (product code: LT27-040).

Very high levels of AK

In some experimental protocols the cells undergo extensive cytolysis resulting in a very high level of AK leakage. If this is suspected it may be advisable to reduce the amount of AK supplied to the assay by sampling a smaller volume of supernatant from the culture wells.

Ensuring optimal performance

The optimal working temperature for all reagents is 22°C. If reagents have been refrigerated always allow time for them to reach room temperature before use.

Pipettes

As with all assays involving manual pipetting, in order to gain maximal accuracy and to reduce variability, pipettes should be calibrated regularly.

All trademarks herein are marks of Lonza Group or its subsidiaries.
Developed by Lonza Nottingham, Ltd.

Lonza

Transfer of samples
When supernatant samples are used or cells are cultured in a luminescence incompatible format, a transfer step is required (protocols 2 and 4). Extensive research has shown that there is no loss of sensitivity with the assay so long as accuracy in pipetting is maintained.

If prolonged culture of the sample has resulted in the evaporation of media this may increase the concentration of AK in these wells.

If scientific support is required please contact scientific.support@lonza.com

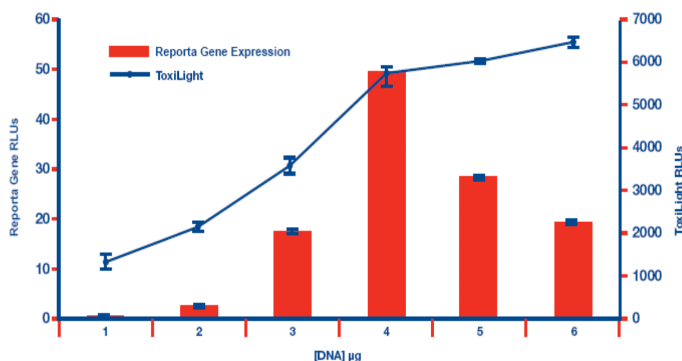


Figure 1: Cytotoxicity detection in transfected cells. Human Umbilical Vein Endothelial Cells (HUVECS) were transiently transfected with increasing amounts of pGL3 luc+ vector for 24 hours. Luciferase activity was measured and cytotoxic effects were detected using the ToxiLight™ kit, showing that a drop in measurement of luciferase activity was associated with adverse effects of the procedure on the cell viability. The combination of these two assays allowed for determination of transfection efficiency. The results are the means of triplicate data points ± SD.

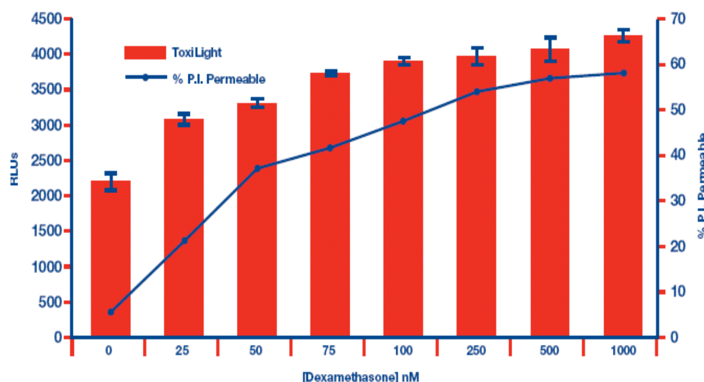


Figure 2: The cytotoxic effect of increasing concentrations of dexamethasone on CEM-7 cells. CEM-7 cells were exposed to dexamethasone for 72h prior to measurement using the AK detection reagent. The results represent the means of triplicate wells ± SD. Comparative data was gained using propidium iodide permeability and flow cytometry.

Ordering information

ToxiLight™ bioAssay kit

LT07-217	500 test kit
LT07-117	1000 test kit

ToxiLight™ bioassay kit with TC plates

LT17-217	500 test kit w/ 5 white TC plates

Related products

ToxiLight™ 100% lysis reagent set
LT07-517 200 Test Kit

White walled clear bottom 96 well TC plates
LT27-102 25 Plates

All trademarks herein are marks of Lonza Group or its subsidiaries.
Developed by Lonza Nottingham, Ltd.