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ViaLight™ plus kit

High sensitivity cell proliferation/cytotoxicity kit with extended signal stability

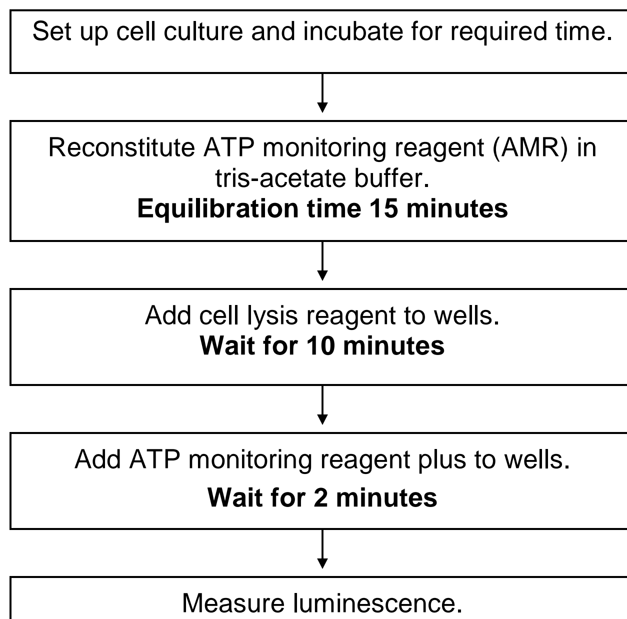
Safety

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

ViaLight™ plus assay procedure

(For detailed assay protocol see specific protocol pg 3)



Kit contents

LT07-221 500 tests; (Sufficient for 5 plates)
1. LT27-212 ATP monitoring reagent plus; Lyophilized; 1 vial
2. LT27-079 Assay buffer; 1 x 50 ml bottle
3. LT27-076 Cell lysis reagent; 1 x 50 ml bottle

LT17-221 500 tests; (Sufficient for 5 plates)
1. LT27-212 ATP monitoring reagent plus; Lyophilized; 1 vial
2. LT27-079 Assay buffer; 1 x 50 ml bottle
3. LT27-076 Cell lysis reagent; 1 x 50 ml bottle
4. 5 x 96 well white walled microplates

LT07-121 1000 tests; (Sufficient for 10 plates)
1. LT27-212 ATP monitoring reagent plus; Lyophilized; 2 vials
2. LT27-079 Assay buffer; 2 x 50 m bottles
3. LT27-076 Cell lysis reagent; 1 x 50 ml bottle

LT07-321 10000 tests; (Sufficient for 100 plates)
1. LT27-213 ATP monitoring reagent plus; Lyophilized; 10 vials
2. LT27-080 Assay buffer; 10 x 100 ml bottles
3. LT27-077 Cell lysis reagent; 5 x 100 ml bottles

The kit should be stored at 2℃-8℃. See kit label for expiration date of the whole kit. See bottle labels for expiration dates of individual components.

Intended use

The ViaLight™ plus kit is intended for the rapid and safe detection of proliferation and cytotoxicity of mammalian cells and cell lines in culture by determination of their ATP levels. ATP (adenosine triphosphate) can be used to assess the functional integrity of living cells since all cells require ATP to remain alive and carry out their specialized functions. The kit can be used for the direct assessment of cell numbers as each individual cell contains ATP. ATP can be detected by the assay thus making it a substitute for tritiated thymidine uptake and tetrazolium dye reduction in cell proliferation assays. Any form of cell injury results in a rapid decrease in cytoplasmic ATP levels and the ViaLight™ plus kit may therefore be used to replace a wide range of endpoint measurements in cell viability testing.

The ViaLight™ plus kit offers many advantages over conventional methods by avoiding the use of radioisotopes, by giving greater reproducibility and higher sensitivity, and by being very rapid. In addition, the kit has been formulated to be used with a microtitre plate reading luminometer for full automation of the assay. The kit can also be used with microplate beta counters and tube luminometers.

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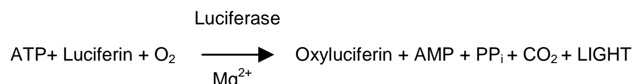
Principles

The kit is based upon the bioluminescent measurement of ATP that is present in all metabolically active cells. The bioluminescent method utilizes an enzyme, luciferase, which catalyses the formation of light from ATP and luciferin according to the following reaction:



The emitted light intensity is linearly related to the ATP concentration and is measured using a luminometer or beta counter. The assay is conducted at ambient temperature (18℃-22℃), the optimal temperature for luciferase enzymes. Bioluminescence is now the most widely used method for the assay of ATP due to its very high sensitivity, wide dynamic range, and ease-of-use.

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.
- Recommended culture volume:
  100 µl per well in a 96 well format.
  25 µl per well in a 384 well format.

1. Add Cell Lysis Reagent to extract ATP from cells.
2. Wait 10 minutes to allow full extraction.
3. Add ATP monitoring reagent plus (AMR plus) to generate luminescente signal.
4. Wait 2 minutes to allow full signal development.
5. 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 cell culture plates compatible with luminescence detection (usually opaque, white walled with clear bottoms?)

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

	Protocol	
	96 Well	384 Well
Luminescence compatible	1	3
Luminescence incompatible	2	4

Reagent reconstitution and storage

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

The ATP monitoring reagent plus (AMR plus) is supplied as a lyophilized pellet. This is reconstituted in assay buffer (supplied) to produce the working solution for use in the assay.

1. Preparation of ATP monitoring reagent plus (AMR plus)

For 96 and 384 well plate:

- Add assay buffer into the vial containing the lyophilized AMR plus until the vial is approximately 75% full.
- Replace the yellow screw cap and mix gently.
- Pour the reconstituted reagent into the remaining assay buffer.
- Repeat the above process to ensure all the lyophilized reagent has been transferred into the assay buffer.
- Allow the reagent to equilibrate for 15 minutes at room temperature to ensure **complete** rehydration.

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

2. Cell lysis reagent

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

3. Assay buffer

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

Equipment

1. Instrumentation

The ViaLight™ plus 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℃, the optimal temperature for luciferase activity.

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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)

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, or opaque white microtitre plates suitable for luminescence measurements.

NOTE: The same microplates should be used with beta counters).

c) Multichannel micropipettes –
50-200 µl (96 well plates)
5-50 µl (384 well plates)

*These plates are supplied as part of a 5 plate ViaLight™ plus kit by Lonza (product code LT17-221) or as a separate product (product code LT27-102; 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 for the correct protocol selection.

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

Protocol 1: Adherent/suspension cells
Luminescence compatible plate
96 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AMR plus in assay buffer. 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 a 1 second integrated reading of each appropriate well.
5. Add 50 µl of cell lysis reagent to each well and wait at least 10 minutes.
6. Add 100 µl of AMR plus to each appropriate well and incubate the plate for 2 minutes at room temperature.
7. Place plate in luminometer and initiate the program.

Protocol 2: Adherent/suspension cells
Luminescence incompatible plate
96 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AMR plus in assay buffer. 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 a 1 second integrated reading of each appropriate well.
5. Add 50 µl of cell lysis reagent to each well and wait at least 10 minutes.
6. Transfer 100 µl of cell lysate to a white walled luminometer plate.
7. Add 100 µl of AMR plus to each appropriate well and incubate the plate for 2 minutes at room temperature.
8. Place plate in luminometer and initiate the program.

Protocol 3: Adherent/suspension cells
Luminescence compatible plate
384 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AMR plus in assay buffer. 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 a 1 second integrated reading of each appropriate well.
5. Add 10 µl of cell lysis reagent to each well and wait at least 10 minutes.
6. Add 25 µl of AMR plus to each appropriate well and incubate the plate for 2 minutes at room temperature.
7. Place plate in luminometer and initiate the program.

Protocol 4: Adherent/suspension cells
Luminescence incompatible plate
384 well format

1. Bring all reagents up to room temperature before use.
2. Reconstitute the AMR plus in assay buffer.

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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 a 1 second integrated reading of each appropriate well.
5. Add 10 µl of cell lysis reagent to each well and wait at least 10 minutes.
6. Transfer 25 µl of cell lysate to a white walled luminometer plate.
7. Add 25 µl of AMR plus to each appropriate well and incubate the plate for 2 minutes at room temperature.
8. Place plate in luminometer and initiate the program.

Interpretation of results

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

Different culture media may quench the light output from the bioluminescent reaction to differing degrees. When comparing results from cultures grown in different media, it is advisable to express the data in terms of cellular ATP concentration. An ATP standard is available separately (product code LT27-008). This can be used to generate a standard curve to which all samples can then be referred. Due to the wide dynamic range of the ATP assay, the standard curve may cover the range from 1×10^{-11} M to 1×10^{-6} M in the reaction mixture. It is important to dilute the standard samples with the appropriate fresh complete culture medium.

The reaction mixture used for standard curves should consist of 100 µl ATP dilution, 50 µl of cell lysis reagent and 100 µl AMR (reconstituted in assay buffer) in a 96 well plate; or 25 µl ATP dilution, 10 µl of cell lysis reagent and 25 µl AMR plus (reconstituted in assay buffer) in a 384 well plate.

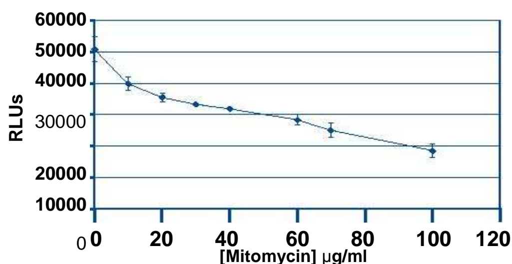

Figure 1: The effect of mitomycin C exposure on CHO cells. CHO (Chinese hamster ovary) cells were seeded into a 384 well plate and treated with varying concentrations (0-100 µg/ml) of Mitomycin C for 48 h. The effect of this alkylating agent was assessed using the ViaLight™ Plus Kit. The data shown is the mean of 12 replicates ± SD.

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Troubleshooting

High background levels?

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 equivalent.

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

Ensuring optimal performance

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

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Integral read time

Reproducibility can be increased by extending the read time from 1 second to a maximum of 10 seconds. However, It should be noted that this will significantly increase the time taken to read the plate.

Shaking

Following the addition of reagents containing cell lysis reagent we recommend that the plates are not shaken as this will induce frothing. The bubbles produced may deflect the light signal away from the detection unit, reducing the number of RLUs observed and producing an artificially low result.

Pipettes

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

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 as long as these are carried out as accurately as possible.

If prolonged culture of the sample has resulted in evaporation of media and the total transfer volume is less than that recommended in the protocol, as long as an equal volume of lysate is transferred from all wells results should still be accurate.

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

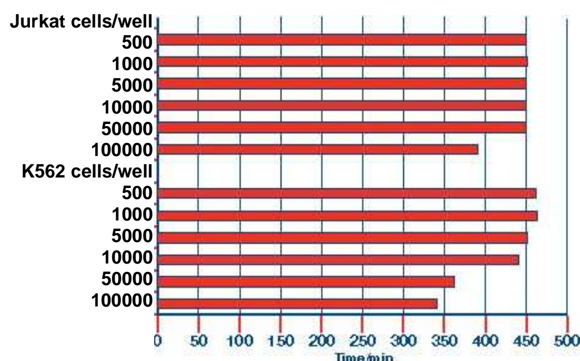

Figure 2: Signal half life. Varying numbers of K562 cells or Jurkat cells were plated out at the cell numbers shown. The cells were assayed using the ViaLight™ plus kit following the standard protocol. The signal generated was monitored every 10 minutes to assess the rate of signal decay. The point at which the signal had decayed to 50% of the original RLU was determined as the signal half life. (See also Figure 3).

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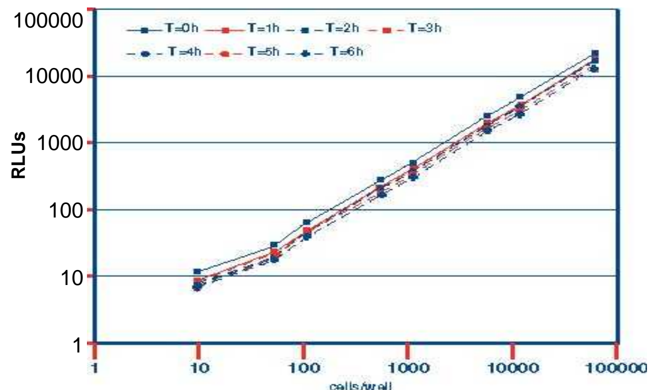

Figure 3: Signal Stability and Linearity of Detection. Varying cell numbers of Jurkat cells were seeded and the luminescent signal was monitored hourly over a 6 hour period after addition of reagent. Typical R^2 values > 0.99.

Ordering information

ViaLight™ plus bioassay kit

LT07-221	500 test kit
LT07-121	1000 test kit
LT07-321	10000 test kit

ViaLight™ plus bioassay kit with TC plates

LT17-221	500 test kit w/ 5 white TC plates

Related products

ATP standard

LT27-008	5 ml

White walled clear bottom 96 well TC plates

LT27-102	25 plates