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ViaLight™ MDA plus

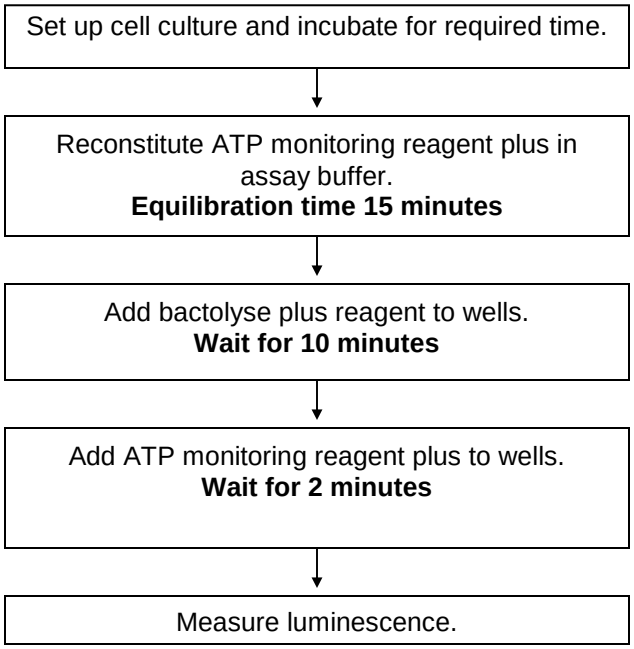
High sensitivity microbial detection kit with extended signal stability

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™ MDA plus assay procedure
(For detailed assay protocol see pg 2)



Kit contents

It is advisable to employ good laboratory practice when handling all kit components.

The kit contains all the required reagents to perform the assay.

LT07-122 1000 tests; (Sufficient for 10 plates)
1. LT27-212 ATP monitoring reagent plus; Lyophilized; 2 vials
2. LT27-079 Assay buffer; 2 x 50 ml bottles
3. LT27-240 Bactolyse plus; 1 x50 ml bottle

LT07-322 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. LT27-241 Bactolyse plus; 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™ MDA plus kit is intended for the rapid enumeration of viable microbial cell numbers. The kit utilizes ATP bioluminescence, the most rapid method of detecting living microbes, facilitating the detection of low cell numbers. The kit can be used to quantify the number of viable cells in many applications both in the research and routine laboratory, and can be used to measure both microbial growth and toxicity.

The ViaLight™ MDA plus kit offers many advantages over conventional methods by being very rapid, sensitive and highly reproducible. In addition, the kit is formulated for use with microtitre plate reading luminometers to provide for full automation of the assay.

The kit can also be used with microplate beta-counters and tube luminometers.

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 catalyzes the formation of light from ATP and luciferin according to the following reaction:

$$\text{ATP+ Luciferin + } O_2 \xrightarrow[Mg^{2+}]{\text{Luciferase}} \text{Oxyluciferin + AMP + } PP_i + CO_2 + \text{LIGHT}$$

The emitted light intensity is linearly related to the

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ATP concentration and is measured using a luminometer or beta counter. The assay is conducted at ambient temperature (18˚C-22˚C), the optimal temperature for insect enzymes. Bioluminescence is now the most widely used method to measure ATP due to its high sensitivity, wide dynamic range, and ease of use.

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 equilibration time.

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)

- 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˚C-8˚C. Unused reagent can be aliquoted into polypropylene tubes and stored at -20˚C for up to 2 months. Once thawed, reagent must not be refrozen and reagents should be allowed to reach room temperature before use without the aid of artificial heat.

2. Bactolyse plus
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™ MDA 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.

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) Opaque white microtitre plates suitable for luminescence measurements
The same microplates should be used with beta counters
c) Multichannel micropipettes -50 µl-200 µl (96-well plates)

Protocol

NOTE: 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.

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.

1. Bring all reagents up to room temperature before use.
2. Reconstitute the ATP monitoring reagent plus (AMR plus) in assay buffer. Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Allow the culture vessel 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. Transfer 100 µl of cell suspension to a white walled luminometer plate. *(If cells are currently in luminescent compatible plate, skip step.)*
6. Add 50 µl of bactolyse plus to each well and wait at least 10 minutes.
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.

Interpretation of results
The relative light unit (RLU) reading obtained from the luminometer can be used as a direct measure of the viable cell numbers present in the sample. The RLU value can therefore be used directly to determine the effect of agents that interfere with microbial growth in culture.

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 (catalog no. 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 10^{-11} to 10^{-6} M ATP in the reaction mixture. It is important to dilute the ATP 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 bactolyse plus and 100 µl ATP monitoring reagent plus in a 96 well plate.

References
Crouch, S., Kozlowski, R., Slater, K., and Fletcher, J. (1993) The use of ATP Bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods* **160**: 81-88.

Dexter, S.J., Camara, M., Davies, M. and Shakesheff, K.M. (2003) Development of a bioluminescent ATP assay to quantify mammalian and bacterial cell numbers from a mixed population. *Biomaterials* **24:** 27-34.

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 scrupulously 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 (catalog no. LT 27-040).

Ensuring optimal performance
Microbial cultures contain their maximal amounts of ATP per cell when in the log phase of growth. To ensure sensitivity and accurate enumeration of viability it is advised that cells are used during this phase.

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.

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

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

Transfer of samples
When cells are cultured in a luminescence incompatible format a transfer step is required (step 5). 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.

Use of 384-well format
For advice on using the ViaLight™ MDA plus assay in 384-well formats please contact Scientific Support as shown below.

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

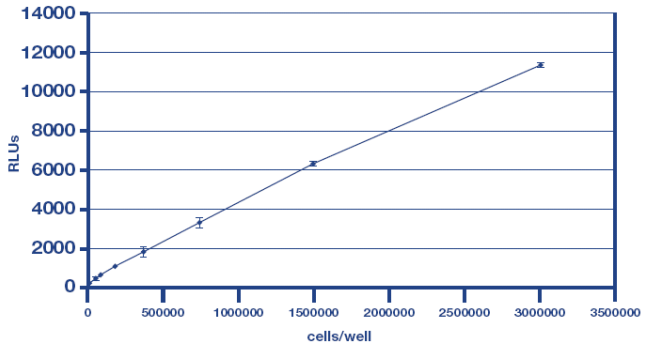

Figure 1: Detection limit. The detection of increasing bacterial (Enterococcus sp.) cell numbers using ViaLight™ MDA plus assay kit. Data is presented as the means of triplicates from one representative experiment. Typical R^2 >0.99.

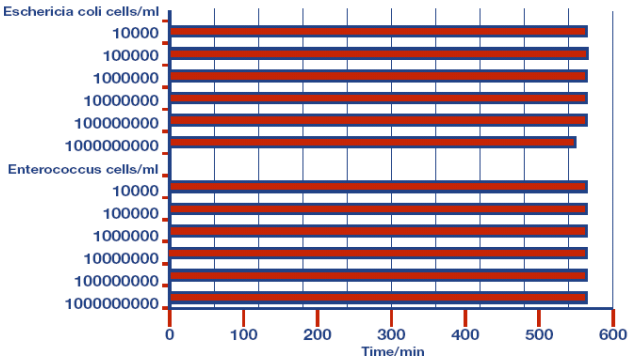

Figure 2: Signal half life. Varying cell numbers of *E.Coli* and Enterococcus sp. were plated out at the cell numbers shown. The cells were assayed using ViaLight™ MDA plus assay kit following the standard protocol. The signal generated was monitored every 6 minutes to assess the rate of signal decay. The point at which the signal had decayed to 50% of the original RLUs was determined as the signal half life. Samples show a typical half life in excess of 9 hours.

Ordering information

ViaLight™ MDA plus bioassay kit

LT07-122	1000 Test kit
LT07-322	10000 Test kit

Related products

ATP standard

LT27-008	5 ml

White walled clear bottom 96 well TC plates

LT27-102	25 Plates