AB-2960 Bactolumix

Introduction

BactoLumix can be used for drug sensitivity test and antibiotic activity test, through the chemiluminescent determination of viable bacteria count.

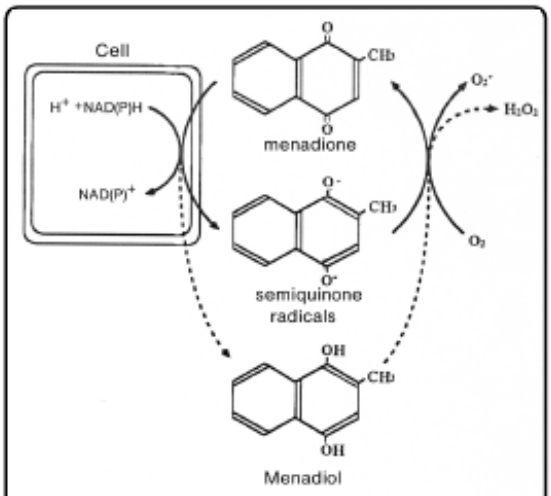


Fig. 5. Proposed mechanism for menadione-catalyzed O_2^- production by *E. coli* cells.

Excerpts from the references listed in end

Principle

This method luminometrically determines the active oxygen generated by the redox reaction between bacterial quinine oxidoreductase (NAD(P)H:) and menadione.

Package (for 1000 samples)

Chemiluminescent Reagent (powder)・・ 1 bottle
Buffer for Dissolution of Chemiluminescent Reagent ・・・1 bottle
Catalyst・・・ 1 bottle
Buffer for Dilution of Catalyst・・・ 1 bottle
Powder Medium for Microbial Culture・・・ 1 bottle

Precautions for Use

Please read following notes for maximizing the performance of BactoLumix.
1. BactoLumix is for research use only. Do not use this product for any other purpose.
2. This kit should be stored in a refrigerator. Upon receipt, immediately store the product in an appropriate place.
3. Do not use products that have expired.
4. When estimating viable bacteria count, make a standard curve using the microbe of interest and medium to be used.
5. When a culture medium not included in this kit is used, the background for luminescent measurement may be influenced. Measure the background derived from the culture medium in advance.
6. When a culture medium not included in this kit is used for proliferating bacteria cells and for making dilution series or counting of viable bacteria, the background for luminescent measurement may be influenced. Measure the background derived from the culture medium in advance.
7. The catalyst should be mixed with "Buffer for Dilution of Catalyst" just before use. The catalyst mixed with the buffer cannot be stored.
8. Filter sterilization of "Medium for Microbial Culture" after preparation is not recommended. Such operation may cause high background, resulting in the difficulty in microassay.
9. Contamination of "Buffer for Dilution of Catalyst" by microorganisms may cause false results. Handle the buffer aseptically.

Procedure

1. Pour the whole bottle of "Buffer for Dissolution of Chemiluminescent Reagent" to the bottle of "Chemiluminescent Reagent," and desolve it completely with a stirrer for approximately 10 minutes.
2. Dissolve "Powder Medium for Microbial Culture" in 300 mL of distilled water (or pure water), and autoclave the solution (at 116-121°C for 10 minutes).
Note:
Filter sterilization is not allowed. It causes high background, which may inhibit microassay.

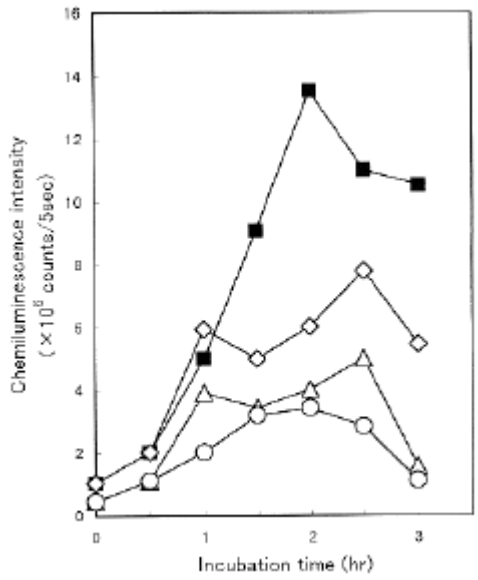
3. Dissolve "Catalyst" in 10 mL of ethanol (special grade).
4. Mix 10 mL of "Buffer for Dilution of Catalyst" with 0.1 mL of "Catalyst" in ethanol aseptically. This becomes "Catalyst Mixture Solution."

Notes:
"Catalyst Mixture Solution" cannot be stored. Prepare just before use.
"Buffer for Dilution of Catalyst" should be handled aseptically.
Confirm that the buffer is not contaminated by observing the turbidity of solution, before use.

Recommended Measurement Apparatus
Microplate-type luminometer (Atto Corporation)
AB2350 Luminescencer PHELIOS
Tube-type luminometer (Atto Corporation)
AB-2270 Luminescencer OCTA

Measurement Procedure (Outline)
1. Pregrow the bacteria of interest in "Medium for Microbial Culture."
2. Treat the bacterial culture, according to purpose.
e.g.) Sensitization with antibiotics, heat sterilization
3. Put 50 μL of the treated bacterial suspension in a container for measurement.
4. Add 50 μL of "Catalyst Mixture Solution" to the container.
5. Incubate the bacteria and the catalyst.
Appropriate incubation time and temperature should be selected depending on bacterial strain.
6. In the container, put 100 μL of "Chemiluminescent Reagent" and measure luminescence intensity for 2 seconds in a luminescence analyzer.

Effect of Antibiotics (Protein Synthesis Inhibitors) on Escherichia coli



■Control
◇Erythromycin (20 μg/mL)
△Tetracycline (2 μg/mL)
○Oxytetracycline (2 μg/mL)

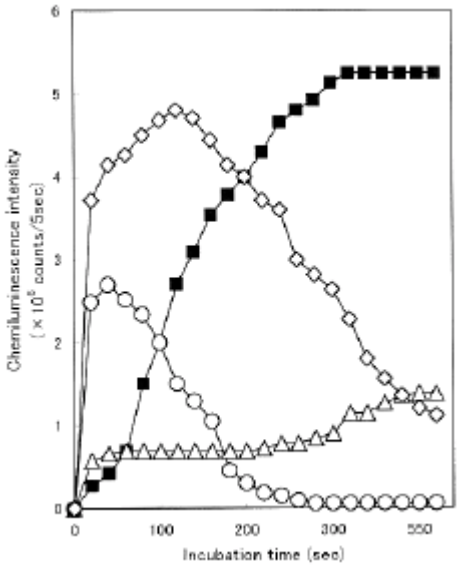
Protein synthesis inhibitors showed delayed bactericidal effects as compared with membrane function inhibitors, however, the significant bactericidal effects were seen 2 hours after addition to Escherichia coli.

Micrbiol. Immunol. 45(5)333-340 (2001) Fig. 4

Specific Examples
Drug sensitivity testing
1. Preculture the bacteria of interest in "Medium for Microbial Culture."
2. Add the test drug to a certain number of bacteria, and incubate it for sensitization.
Incubation time is several ten seconds for drugs inhibiting membrane function or 4 - 6 hours for drugs inhibiting the synthesis of DNA / RNA, proteins or peptidoglycan.
3. Take 50 μL aliquot of the sensitized culture into a measurement container. .
4. Add 50 μL of "Catalyst Mixture Solution" to the measurement container, and incubate it for approximately 10 minutes.
5. Add 100 μL of "Chemiluminescent Reagent" to the measurement container and measure luminescence intensity for 2 seconds with luminometer.
6. Evaluate the sensitivity of the bacteria to the drug by comparing the measured luminescence

intensity between with and without the drug.
* Exchanging of medium for measurement is unnecessary.
* For estimating viable bacteria count from the measured luminescence intensity, a standard curve should be made for each bacteria at each assay run.
* An example of the results of drug sensitivity testing is shown below.

Effect of Membrane Function Inhibitors on Escherichia coli



■Control
◇Polymyxin B (5 μg/mL)
○Benzalkonium chloride (50 μg/mL)
△Carbonylcyanide
Chlorophenylhydrazone (0.2 μg/mL)

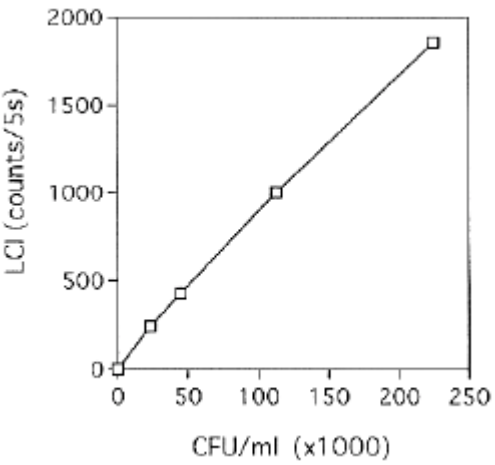
The bactericidal effects were seen within 10 minutes after addition to Escherichia coli.

Microbiol. Immuno. l. 45(5)333-340 (2001) Fig. 3

Viable Bacteria Counting

1. Culture the bacteria of interest in “Medium for Microbial Culture.”
2. Dilute the bacterial culture serially with “Medium for Microbial Culture.”
3. Take each 50 μL of samples and the serially diluted bacterial culture into measurement containers
4. Add 50 μL of “Catalyst Mixture Solution” to the container and incubate it for approximately 10 minutes.
5. Add 100 μL of “Chemiluminescent Reagent” to each container and measure luminescence intensity for 2 seconds with luminometer.
6. Make a standard curve from the serial dilutions of bacteria.
7.Estimate the viable bacteria count in the samples from the standard curve.

* For Pseudomonas aeruginosa, luminescence intensity cannot be obtained according to growth condition.
* Exchanging of culture medium for measurement is unnecessary.
* The time from the addition of Chemiluminescent Reagent through measurement may vary depending on bacteria.
* For estimating viable bacteria count from the luminescence intensity, a standard curve should be made for each bacteria at each assay run.



Viable bacteria counting of Escherichia coli during log phase growth
To the microbial suspension that had been incubated with menadione for 10 minutes, Chemiluminescent Reagent was added and luminescence intensity was measured.

Disposal

The containers of Chemiluminescent Reagent, Buffer for Dissolution of Chemiluminescent Reagent and Powder medium for Microbial Culture are made of the following materials.

Chemiluminescent Reagent/ Buffer for Dissolution of Chemiluminescent Reagent
- Bottle: High density polyethylene
- Inside plug: Linear low density polyethylene
- Lid: Polypropylene

Catalyst
- Bottle: Soda glass
- Lid: Polypropylene

Buffer for Dilution of Catalyst
- Bottle: Polystyrene
- Lid: High density polyethylene

Powder Medium for Microbial Culture
- Bottle and lid: Polypropylene

Used reagents and bacteria should be disposed according to the laboratory standard operation procedure, and the lows and regulations of local authorities.

Storage

1. “Chemiluminescent Reagent” is stable in a refrigerator (at 4°C) until mentioned expiration date, avoiding light. “Chemiluminescent Reagent” dissolved in buffer can be used for 6 months, when it is stored at 4°C avoiding light.
2. “Catalyst” dissolved in ethanol is stable at -20°C for 6 months. When stored 4°C, it is stable for 3 months.
3. “Buffer for Dilution of Catalyst” is stable at 4°C for 1 year.
4. Dissolved “Powder Medium for Microbial Culture” is stable at 4°C for 1 month.
5. The reagent is generally shipped at room temperature. Although storage at room temperature for 2 or 3 days does not affect the quality of the product, store it at 4°C as early as possible after receipt.

References

1) Shiro Yamashoji , Isao Manome , and Masanari Ikedo (2001) Menadione-Catalyzed O_2^- Production by *Escherichia coli* Cells:Application of Rapid Chemiluminescent Assay to Antimicrobial Susceptibility Testing. Microbiol. Immunol. , 45(5) , 333-340

2) Shiro Yamashoji , and Mitsue Takeda (2001) Menadione-Catalyzed Luminol Chemiluminescent Assay for the Viability of *Escherichia coli* ATCC 25922 . Microbiol. Immunol.,45(11),737-741

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