Version 1 Last updated 29 May 2018

ab189820 Bacterial Assay (Luminescent)

For the measurement of bacterial cells in a number of media or biological sources.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Bacterial Assay (Luminescent) (ab189820) provides an easy and quick method to detect and quantify bacterial cells based on ATP present in metabolically active cells. The kit contains a genetically engineered luciferase which generates very stable luminescent signals. This luciferase emits red upon luciferin conversion and thus can be multiplexed with other assays using blue or green fluorescence.

Prepare reagents and Bacterial lysis mix.

Mix bacterial culture sample with bacterial lysis mix for 10 minutes at room temperature.

Transfer the mixture to a 96 well black microtiterplate.

Add Luciferin/Luciferase solution to each well.

Record luminescence with a luminometer or a plate reader with luminescence recording capability.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 6 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature
Luciferin Substrate*	1.4 mg	-20°C
Luciferase	0. 5 mg	-80°C
Lysozyme*	20 mg	-20°C
Reaction Buffer	6 mL	4°C
Lysis Buffer	12 mL	4°C
Phosphate/EDTA Buffer	2 mL	4°C

** Store lyophilized*

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Luminometer or plate reader with luminescence detector.
- 96-well black walled assay plate.
- 96-well clear plate for sample processing in microtiterplate format or microcentrifuge tubes for sample processing in single tube format.
- Multi-channel pipette.
- Dry ice.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

5.1 Luciferin Substrate

1. Ready to use as supplied.
2. Bring the solution to room temperature.

5.2 Luciferase

1. Ready to use as supplied.
2. Keep the thawed vials on ice prior to use.

5.3 Lysozyme

1. Ready to use as supplied.

5.4 Reaction Buffer

1. Ready to use as supplied.
2. Keep the thawed vials on ice prior to use.

5.5 Lysis Buffer

1. Ready to use as supplied.
2. Thaw and store at 4 °C until use.

5.6 Phosphate/EDTA Buffer

1. Ready to use as supplied.
2. Thaw and store at 4 °C until use.

6. Assay Procedure

– Assay all standards, controls and samples in duplicate.

6.1 Reconstitute solutions

1. Transfer exactly 5 mL of the completely thawed Reaction Buffer to the Luciferin Substrate. Swirl or vortex gently to dissolve.
2. Transfer the Luciferase to the substrate solution prepared above. Swirl to mix.
3. To ensure complete transfer, pipet 1 mL of luciferin solution to the luciferase vial to rinse and transfer back to the substrate bottle.

Δ Note: The well mixed solution currently in the substrate bottle is the Luciferin/Luciferase working solution. This working solution should be kept on ice. Any unused solution can be stored at 4°C and will remain active for approximately 2 weeks. Alternatively, unused portions can be aliquoted and stored at -20°C for extended periods.

6.2 Measurement of ATP from bacteria

Δ Note: Extracting ATP from bacteria can be performed in a single tube or in a microtiterplate format if multiple samples are required to be processed at the same time.

1. Thaw Phosphate/EDTA Buffer as well as Lysis Buffer and store at 4°C until use.
2. To the Lysozyme vial, add 0.4 mL of Phosphate/EDTA Buffer and 3.6 mL of deionized water. Bring final mixture to complete solution.
3. Transfer this 4 mL solution to the Lysis Buffer. Swirl to mix.
   Δ Note: This lysis mixture is called "Bacterial Lysis Mix" and can be used to lyse and extract ATP from bacteria.
4. Transfer 45 µL of bacterial culture sample(s) to a tube or microtiterplate well containing 5 µL of Phosphate/EDTA Buffer.
5. Mix well by pipetting up and down several times, and then place on dry ice to freeze completely. (Include control wells containing only culture medium to obtain a value for any background luminescence).
6. Thaw the mixture to room temperature and add 150 µL of Bacterial Lysis Mix prepared in step 6.2.2.
7. Allow the mixture to stand at room temperature for 10 minutes. The total lysate volume will be 200 µL.

8. Transfer 50 µL of this cell lysate to a 96-well black-walled flat or round bottom microtiterplate.
   Δ Note: Please consult your microtiterplate reader's instruction manual for the appropriate plate to use.
9. Dispense 50 µL of the Luciferin/Luciferase working solution to each well, mix and record luminescence [using a time for integration of ≥ 1 second] with a luminometer or a plate reader with luminescence recording capability.

7. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

8. Typical Data

Data provided **for demonstration purposes only.**

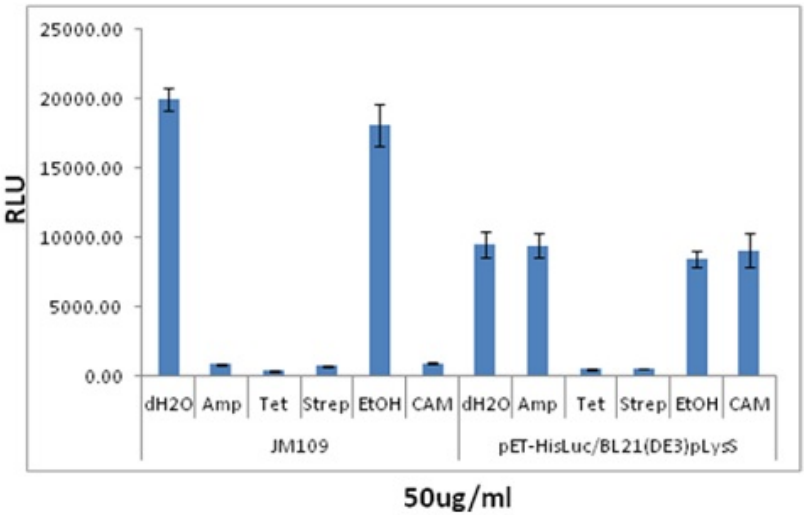

Figure 1. Evaluating the growth inhibition of bacteria by antibiotic reagents: Cultures from JM109 or BL21(DE3)pLysS (chloramphenicol resistance) strains harboring an expression vector (confer ampicillin resistance) were diluted and mixed with a number of antibiotics. This mixture was incubated at 37°C for 5 hours. Then cells were lysed, ATP extracted and measured with ab189820 Bacterial Assay (Luminescent). Luminescence was recorded. RLU was plotted against the different antibiotics. AMP:ampicillin; Tet:tetracyclin; Strep:streptomycin; CAM:chloramphenicol. Ampicillin and chloramphenicol inhibited the growth of JM109, but didn't affect the growth of resistant strain.

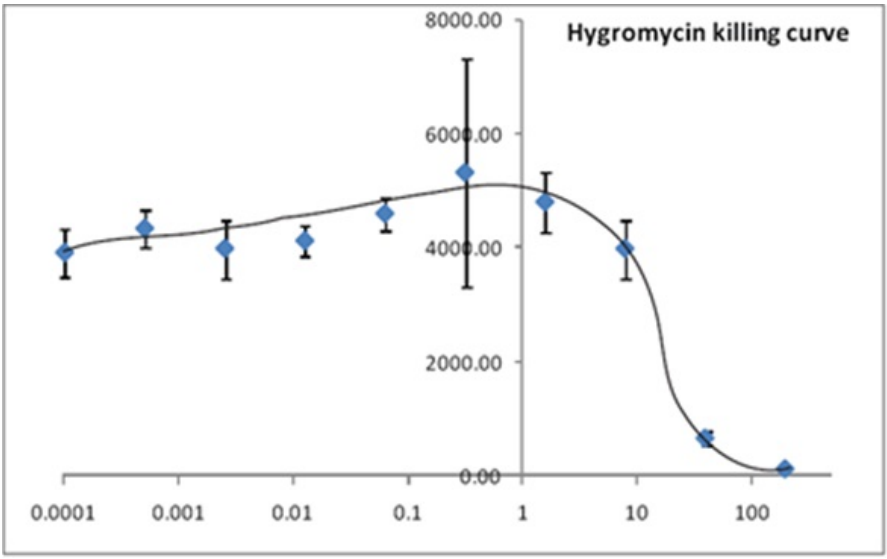

Figure 2. Dose-response growth inhibition of bacteria by Hygromycin: Cultures from JM109 were diluted and mixed with a series of Hygromycin dilutions. This mixture was incubated at 37°C for 5 hours, lysed, ATP extracted and measured with ab189820 Bacterial Assay (Luminescent). Luminescence was recorded. RLU was plotted against Hygromycin concentration.

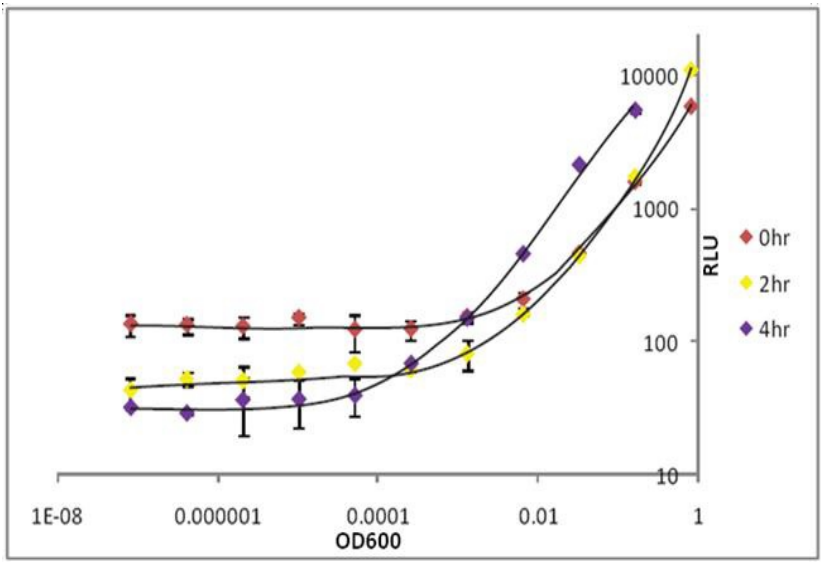

Figure 3. Detection limit of bacteria by ab189820 Bacterial Assay (Luminescent): Dilutions from exponentially grown W3110 were lysed, ATP extracted and luminescence recorded. To examine whether extended incubation at 37°C will help the detection of high dilution culture, we tried 2 and 4 hour incubation periods and found that the detection limit was dropped at least two magnitudes.

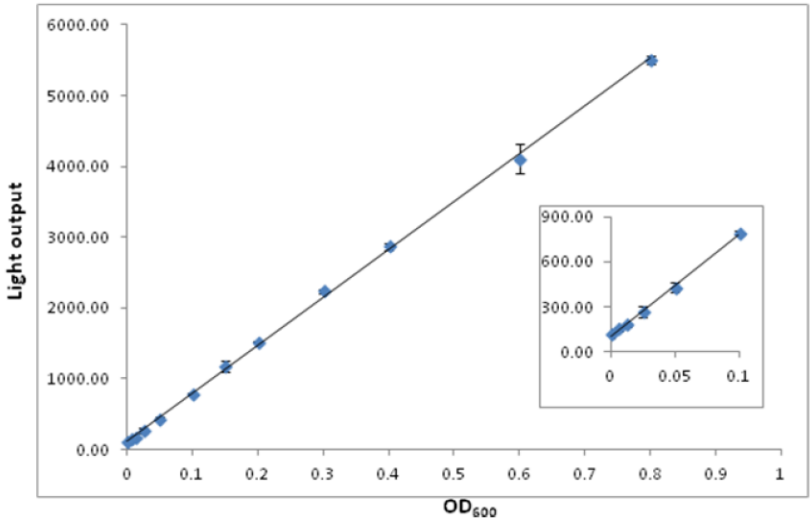

Figure 4. Linearity of luminescence vs growth of bacteria: Dilutions from exponentially grown W3110 were lysed, ATP extracted and luminescence recorded. RLUs were plotted against the bacterial growth which was indicated by OD_{600}. Linearity was observed up to OD_{600} of 0.8. The insert shows the linearity below OD_{600} of 0.1.

9. Notes

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