



Promega

Technical Bulletin

ENLITEN[®] Total ATP Rapid Biocontamination Detection Kit

INSTRUCTIONS FOR USE OF PRODUCT FF3710.



www.promega.com

ENLITEN[®] Total ATP Rapid Biocontamination Detection Kit

All technical literature is available on the Internet at: www.promega.com/tbs/
Please visit the web site to verify that you are using the most current version of this
Technical Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail: techserv@promega.com

1. Description	1
2. Product Components and Storage Conditions	2
3. Summary and Explanation.....	3
4. Preparation for the Assay	4
A. Preparation of rL/L Reagent	4
B. Performance Check	4
C. Instrument Preparation	5
5. Protocols	5
A. Performing the Assay Using a Luminometer Without Injectors (Manual Method).....	5
B. Performing the Assay Using a Luminometer with Injectors (Automated Method).....	6
6. Interpretation of Results	7
7. ENLITEN [®] Total ATP Rapid Biocontamination Detection Kit Limited Warranty	8

1. Description

Intended Use

The ENLITEN[®] Total ATP Rapid Biocontamination Detection Kit^(a) is intended to test surfaces for biocontamination by detecting ATP that may result from food residue, microbes or other biological sources.

Limitations of the Procedure

The ENLITEN[®] Total ATP Rapid Biocontamination Detection Kit is not designed to directly test materials such as food products for the presence of microbial contaminants and is not designed for any clinical diagnostic application.

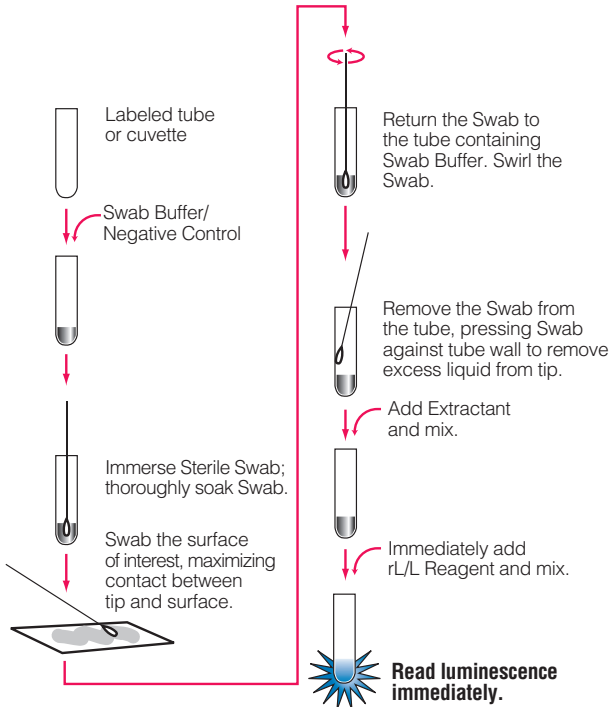


Figure 1. Summary of the ENLITEN® Total ATP Rapid Biocontamination Detection Assay.

2. Product Components and Storage Conditions

Product	Size	Cat.#
ENLITEN® Total ATP Rapid Biocontamination Detection Kit	100 determinations	FF3710

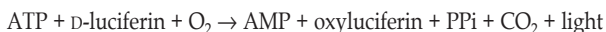
Each system contains sufficient reagents for 100 determinations. Includes:

- 1 vial rLuciferase/Luciferin (rL/L) Reagent
- 1 vial rL/L Reconstitution Buffer
- 1.5 ml Positive Control
- 12 ml Swab Extractant
- 4 vials Swab Buffer/Negative Control
- 100 Sterile Swabs (in separate package)

Storage Conditions: Store the entire kit at -20°C or as per individual component labels.

3. Summary and Explanation

The light from fireflies is produced by an enzyme called luciferase, which catalyzes an ATP-dependent oxidation of luciferin. This reaction is shown below:



Since all living cells rely on ATP for metabolic energy, the detection of ATP can be used as an indication of the presence of living microorganisms, food residues or other sources of cellular debris. The ENLITEN® Total ATP Rapid Biocontamination Detection Kit utilizes the ATP-dependence of the firefly luciferase reaction to detect the presence of biological material (including microbial cells or food residues) on surfaces.

The luciferase in this kit is produced using recombinant DNA technology to provide a higher degree of reproducibility and reliability.

Measuring ATP levels in the environment provides a system to monitor contamination and cleanliness. To use the ENLITEN® Total ATP Rapid Biocontamination Detection Kit, sample the surface using a sterile swab. Immerse the swab in Swab Buffer, add the Extractant, then the rL/L (Luciferase/Luciferin) Reagent. Within the linear range of the assay, the light produced is proportional to the amount of ATP on the swab, which is measured immediately using a luminometer (Figure 1). Sample results are compared to a threshold level of contamination established by the user. Detailed protocols are given in Section 5. A general outline of the procedure is shown in Figure 1.

Materials to be Supplied by the User

- micropipettes capable of delivering from 0.1ml to 1ml
- luminometer
- cuvettes or tubes compatible with the luminometer
- cuvette rack
- nitrile, vinyl or latex gloves

Note: Due to latex sensitivity and allergies, certain individuals may not be able to use latex gloves. For those individuals, we recommend hypoallergenic nitrile or vinyl gloves.

4. Preparation for the Assay

Warning: Sodium azide is included in the rL/L Reconstitution Buffer. Avoid contact with this solution, as it may be harmful if swallowed or absorbed through the skin. If the reagent comes into contact with exposed skin or eyes, wash the affected area with water for several minutes.

Gloves should be worn during the reconstitution of the rL/L Reagent and while performing the assay to protect against contamination.

4.A. Preparation of rL/L Reagent

1. Gently tap the vial of rL/L Reagent (Part# F120A) before opening to ensure that all dried material is on the bottom of the vial.

Caution: Do not touch the bottom of the rubber stopper with your bare fingers. ATP contamination from your hands could cause erroneous results. Gloves should be worn during the reconstitution step to avoid contamination of the reagent.

2. Transfer the contents of the vial of rL/L Reconstitution Buffer to the vial of rL/L Reagent.
3. Replace the stopper carefully, and gently invert the vial several times to dissolve the contents. **Do not shake the dissolved rL/L Reagent.**
4. Allow the reconstituted rL/L Reagent to stand for 1 hour at room temperature before use.

After the reconstitution step is completed, the prepared rL/L Reagent may be kept for a maximum of one day at room temperature (20–25°C). However, slight decreases in Positive Control RLU values are expected due to gradual decline in luciferase activity.

For storage beyond 5 days: Freeze aliquots of prepared rL/L Reagent immediately following the one-hour incubation step. Avoid multiple freeze-thaw cycles.

4.B. Performance Check

Verify that the luminometer and kit reagents are functioning properly each day that the ENLITEN® Total ATP Rapid Biocontamination Detection assay is performed. Because luminometers vary in sensitivity and different manufacturers use different scales to measure relative light units (RLU), Promega recommends that you establish a control chart for the Negative Control and Positive Control values you obtain.

Expected RLU Values

The **Negative Control** should have a reading near the lower limit of the luminometer you use. If the Negative Control has a high reading, try using an unopened tube of the Swab Buffer/Negative Control.

The **Positive Control** should have a reading at least 100-fold greater than that of the Negative Control.

4.C. Instrument Preparation

1. Set up the luminometer per the manufacturer's instructions. For all luminometers, program the integration time for 10 seconds.
2. Set up automatic injectors as follows:

Luminometers Equipped With One Injector

Install a bottle of reconstituted rL/L Reagent, and prime the injector line. The injector volume should be set to 100 μ l.

Luminometers Equipped With Two or More Injectors

Install the Extractant bottle on the first line and the reconstituted rL/L Reagent on the second line. The injector volume for each should be set to 100 μ l. Program a delay of 1 second between adding Extractant and adding rL/L Reagent.

5. Protocols

5.A. Performing the Assay Using a Luminometer Without Injectors (Manual Method)

Performance Check

Notes:

1. When pipetting, dispense control material directly onto the bottom of the cuvette. Avoid splashing the solution onto the cuvette walls.
2. Assay reagents should be warmed to room temperature (20–25°C) prior to use.

Negative Control: Add 100 μ l of Swab Buffer/Negative Control to a labeled cuvette. Proceed to Step 9.

Positive Control: Add 100 μ l of Positive Control to a labeled cuvette. Proceed to Step 9.

Sample Processing

1. Label one clean tube or cuvette compatible with the chosen luminometer for each determination to be performed.
2. Place the tubes or cuvettes in a holder.
3. Transfer 300 μ l of Swab Buffer/Negative Control to each tube.
4. Immerse a sterile swab in the tube containing Swab Buffer/Negative Control, making certain that the swab is thoroughly soaked.

Note: To avoid contamination, do not touch the tip of the swab with your fingers.

**5.A. Performing the Assay Using a Luminometer Without Injectors
(Manual Method; continued)**

5. Swab the area of interest (an area of approximately $10 \times 10\text{cm}$ is recommended). Twist the handle of the swab between the fingers to maximize contact of the swab tip with the test area.
6. Place the swab back in the appropriately labeled tube/cuvette containing the Swab Buffer.
7. Swirl the swab in the Swab Buffer for 10 seconds. While withdrawing the swab from the cuvette, press the swab tip against the inside cuvette wall to remove as much liquid from the tip as possible.
8. Discard the swab.
9. Add 100 μl of Extractant to each sample, and mix thoroughly.
10. Add 100 μl of rL/L Reagent to each sample. Mix and read the luminescence immediately. Record the RLU reading.

**5.B. Performing the Assay Using a Luminometer with Injectors
(Automated Method)**

Performance Check

Notes:

1. When pipetting, dispense control material directly onto the bottom of the cuvette. Avoid splashing the solution onto the cuvette walls.
2. Assay reagents should be warmed to room temperature (20-25°C) prior to use.

Negative Control: Add 100 μl of Swab Buffer/Negative Control to a labeled cuvette. Proceed to Step 9.

Positive Control: Add 100 μl of Positive Control to a labeled cuvette. Proceed to Step 9.

Sample Processing

1. Label one clean tube or cuvette compatible with the chosen luminometer for each determination to be performed.
2. Place the tubes or cuvettes in a holder.
3. Transfer 300 μl of Swab Buffer/Negative Control to each tube.
4. Immerse a sterile swab in the tube containing Swab Buffer/Negative Control, making certain that the swab is thoroughly soaked.

Note: To avoid contamination, do not touch the tip of the swab with your fingers.

5. Swab the area of interest (an area of approximately 10 × 10cm is recommended). Twist the handle of the swab between the fingers to maximize contact of the swab tip with the test area.
6. Place the swab back in the appropriately labeled tube/cuvette containing the Swab Buffer.
7. Swirl the swab in the Swab Buffer for 10 seconds. While withdrawing the swab from the cuvette, press the swab tip against the inside cuvette wall to remove as much liquid from the tip as possible.
8. Discard the swab.
9. Process each sample as described below:

Luminometers With One Automatic Injector

- a. Add 100µl of Extractant, and mix thoroughly.
- b. Place the cuvette in the luminometer, which will add rL/L Reagent automatically and read the luminescence. Record the RLU reading for each sample.

Luminometers With Two or More Automatic Injectors

Place each cuvette in the luminometer sample compartment. The luminometer will add Extractant and rL/L Reagent automatically, and read the luminescence. Record the RLU reading for each sample.

6. Interpretation of Results

The ENLITEN® Total ATP Rapid Biocontamination Detection Kit allows rapid assessment of whether a surface is contaminated or contamination-free by using a baseline cutoff value. The baseline cutoff value should be determined using data accumulated for your environment (e.g., processing plant).



Baseline cutoff values should be calculated daily.

In the absence of data specific to your environment, the following procedure can be used to define a baseline cutoff value:

$$\text{baseline cutoff value} = \text{Positive Control RLU value} \times 0.02$$

Note: Other baseline cutoff values may be used as needed.

The RLU readings for each sample should be compared to the baseline cutoff value. If the RLU reading for a sample is below the baseline cutoff value, the surface can be considered contamination-free. If the RLU reading for a sample is greater than the baseline cutoff value, the surface is contaminated. Surfaces shown to be contaminated should be properly cleaned and retested until they are shown to be contamination-free by the assay.

7. ENLITEN® Total ATP Rapid Biocontamination Detection Kit Limited Warranty

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

⁶⁾The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

© 1998, 1999, 2000, 2002, 2006, 2009 Promega Corporation. All Rights Reserved.

ENLITEN is a registered trademark of Promega Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.