

MicroSnap™ EB (*Enterobacteriaceae*)



Part Numbers:

- MicroSnap™ EB Incubation Device (MS1-EB)
- MicroSnap™ EB Detection Device (MS2-EB)

Description/ Intended Use:

MicroSnap EB (*Enterobacteriaceae*) is a rapid bioluminogenic test for detection and enumeration of *Enterobacteriaceae* bacteria in a sample providing results in 6 to 8 hours. MicroSnap EB consists of an Incubation Device containing proprietary growth media and a Detection Device containing bioluminogenic reagents in which biomarkers produced by bacteria are measured using a Hygiena™ luminometer.

The two-step test procedure requires a short incubation period followed by a detection step. The greater the number of bacteria in the sample, the higher the biomarker concentration and the more light output. An aliquot of the sample is transferred to the Detection Device, activated, mixed, and measured in a luminometer. The light output is directly proportional to the concentration of bacteria present.

MicroSnap EB can be used to test environmental surfaces, product samples, water, and other filterable liquids.

Required Materials (Not Provided):

- EnSURE™ Touch or EnSURE™ luminometer
- Hygiena™ Dry Block Incubator at 37°C ± 0.5°C

Required Materials for Product Samples:

- Sample bags
- Homogenizing equipment
- Pipette and tips for 1 mL
- Product Sample Diluents:
 - Buffered Peptone Water
 - Maximum Recovery Diluent
 - Butterfields
 - Sterile Water

Table 1: Potential Dynamic Range (Limit of Detection)

Sample Type	CFU Range
Surface	0 – 5,000
1mL Liquid	0 – 5,000
10% w/v Suspension of Solid	0 – 50,000 (bacteria/g) *

*For samples where contamination is outside the ranges detailed in Table 1, then dilutions should be made so that it is within the detectable range of the luminometer:

- 1% suspension will be 1,000 – 500,000 CFU
- 0.1% suspension will be 10,000 – 5,000,000 CFU

Test Procedure:

Instructional Videos:

www.youtube.com/HygienaTV

www.vimeo.com/HygienaTV

Step 1: Incubation

Incubation procedure is described below and is also shown in Step 1 diagrams.

1. Samples Preparations Include:
 - a. Surface Samples – Utilize pre-moistened Incubation Device to sample a 4 x 4 inches (10 x 10 cm) square area. For irregular surfaces, ensure swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample.
 - i. Important swabbing technique tips:
 - ii. Rotate swab while collecting sample to maximize sample collection on swab tip.
 - iii. Apply sufficient pressure to create flex in swab shaft.
 - iv. Swab in a crisscross pattern vertically, horizontally, and in both diagonal directions.
 - b. Liquid – Transfer 1mL of liquid or water samples added directly to Incubation Device.
 - c. Product - Transfer 1mL of 10% (1:10) w/v food homogenate into Incubation Device. Food homogenate should be prepared using industry recommended diluents and standard microbiological procedures (e.g., 50g in 450ml of diluent as used in AOAC validation studies). Other sample sizes should be validated by user.

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Re-attach the swab back into swab tube. Device should look the same as it did when first pulled from the bag.

Activate Incubation Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward.

Separate bulb and swab tube about 1-2 inches from each other, relieving internal pressure, and squeeze bulb to flush all media to bottom of swab tube.

Ensure most of the broth is in bottom of swab tube.

Re-attach swab back into swab tube to firmly seal device. Shake tube gently to mix sample and broth.

Incubate at 37 ± 0.5 °C for 6 to 8 hours. (Refer to Tables 2 – 4 for details).

Step 2: Detection

Detection procedure is described below and is also shown in Step 2 diagrams. Before beginning Step 2, turn on luminometer. If locations have been programmed, select location to be tested.

1. Allow the MicroSnap EB Detection Device to equilibrate to room temperature (10 minutes at 20 – 25 °C).
 - a. Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once.
 - b. This will bring extractant liquid dispersed in tube to bottom of tube.
 - c. Extractant is necessary to facilitate mixing of sample with solution in tube.
2. Transfer sample from Incubation Device to Detection Device using the swab in the Incubation Device as a pipette.
3. Squeeze and release Incubation Device bulb to mix and draw sample into bulb.
4. Aseptically open Incubation Device and open Detection Device by twisting and pulling to remove bulb. Set aside. Insert Incubation Device swab tip 1 inch or 3 cm into top of Detection Device tube and lightly squeeze Incubation Device bulb to trickle 3 drops of sample into tube until volume reaches fill line marked on bottom of Detection Device tube. Avoid adding excess sample above fill line, as this can increase variation of test results.
5. Remaining sample can be returned to Incubation Device for additional testing. Reassemble Incubation Device to original state and return device to incubator. *Note: When testing replicates from same incubated sample, all replicates must be performed within 10 minutes to obtain comparable results.*
6. Activate Detection Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward. Squeeze bulb 3 times to release all liquid to bottom of swab tube.
7. Shake gently for 2 seconds to mix.
8. Immediately insert whole device into luminometer; close lid and holding unit upright, press the button to initiate the measurement. Results will appear after 10 or 15 seconds, depending on the instrument you're using.
 - a. EnSURE™ Touch gives results in 10 seconds. Results are shown in CFU (Colony Forming Units). MicroSnap™ samples can be programmed directly on the luminometer or by using SureTrend Cloud.
 - b. EnSURE™ gives results in 15 seconds. Results are shown in RLU (Relative Light Units). Use SureTrend to program MicroSnap™ samples and set RLU thresholds on the EnSURE™ to correspond with required CFU limits. Refer to "Interpretation of Results" below for correlation.

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Interpretation of Results:

Results on EnSURE™ are displayed as Relative Light Units (RLU). RLU is proportional to the starting inoculum and corresponding bacteria equivalent numbers (expressed as Colony Forming Units, CFU). Tables 2 – 4 show the equivalent CFU values for RLU measurements at various incubation times for EnSURE™. This will tell you how many *Enterobacteriaceae* were present in original sample.

Results on EnSURE™ Touch are shown in Colony Forming Units (CFU).

Hygiena's luminometers approved for this test have different performance characteristics and sensitivities so interpretation will differ accordingly. EnSURE™ luminometers have a 4-digit RLU output display and results $\geq 10,000$ RLU will be outside display range (See Table 1). EnSURE™ Touch can give qualitative (pass/fail) as well as quantitative results as CFU/g or CFU/mL.

Table 2: Potential Correlation between RLU and CFU at 6 hours on EnSURE™ (not applicable to EnSURE™ Touch)

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1 mL liquid sample	10% suspension of solid sample
<10	<50/mL	<500/g
<25	<120/mL	<1,000/g
<50	<250/mL	<2,500/g
<100	<500/mL	<5,000/g
<250	<1,200/mL	<12,000/g
<500	<2,500/mL	<25,000/g
<1,000	<5,000/mL	<50,000/g
>1,000	TNTC	TNTC

Table 3: Potential Correlation between RLU and CFU at 7 hours on EnSURE™ (not applicable to EnSURE™ Touch)

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1 mL liquid sample	10% suspension of solid sample
<10	<5/mL	<50/g
<25	<12/mL	<100/g
<50	<25/mL	<250/g
<100	<50/mL	<500/g
<250	<120/mL	<1,200/g
<500	<250/mL	<2,500/g
<1,000	<500/mL	<5,000/g
>1,000	TNTC	TNTC

Table 4: Potential Correlation between RLU and CFU at 8 hours on EnSURE™ (not applicable to EnSURE™ Touch)

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1 mL liquid sample	10% suspension of solid sample
<10	Absence	Absence
<25	Absence	Absence
<50	Absence	<25/g
<100	<5/mL	<50/g
<250	<12/mL	<120/g
<500	<25/mL	<250/g
<1,000	<50/mL	<500/g
>1,000	TNTC	TNTC

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On EnSURE™ - when several dilutions are prepared and tested for samples with unknown contamination, the CFU/ g or mL is calculated by multiplying the RLU result by the corresponding dilution factor. Contact a Hygiena representative for details. EnSURE™ Touch makes this easier because the software does the math for you, using the data generated from the AOAC Validation Studies as well as additional internal testing.

Calibration Control:

It is advisable to run positive and negative controls according to Good Laboratory Practice. Hygiena™ offers the following controls:

- Calibration Control Kit (Part # PCD4000)
- CalCheck (Part # CAL)

Storage & Shelf Life:

- Store at 2 – 8 °C.
- Devices have a 12-month shelf life from the date of manufacturing.
- Check expiration date on label.

Disposal:

Disinfect before disposal. MicroSnap devices can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour. Then, they can be placed in the trash. Alternatively, MicroSnap devices may be discarded at a biohazard waste disposal facility.

Safety & Precautions:

1. MicroSnap™ device components do not pose any health risk when used correctly. Used devices confirming positive results may be a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations (see disposal instructions above).
2. Devices are designed for a single use. Do not reuse.
3. Do not use devices after expiration date.
4. Sampling should be done aseptically to avoid cross contamination.
5. Verify proper incubation temperature and time for the test application. In most cases, this will be 6- or 8-hours incubation as specified in the above instructions, unless you've been directed otherwise by Hygiena's R&D team for custom applications which require different incubation times or temperatures.
6. When testing multiple serial dilutions, all dilutions must be prepared and tested simultaneously to obtain linear results.
7. Ensure proper sample dilution so that it can be read within luminometer's dynamic range.
8. Ensure proper incubation temperature and time for the test application.
9. When testing replicates from same sample, all replicates must be performed within 10 minutes to obtain comparable results.
10. When performing comparison testing, sample assays must be started within 10 minutes for comparable results between methods.

Caution & User Responsibility:

1. MicroSnap™ devices have not been tested with all possible food products, food processes, testing protocols or with all possible microorganism strains.
2. Do not use this test for diagnosis of conditions in humans and animals.
3. No single culture medium will recover the same strain or enumerate a particular strain in the same way as another medium. Other external factors such as sampling method, testing protocol, and handling may influence recovery.
4. It is the user's responsibility when selecting a test method to evaluate a sufficient number of samples.
5. As with any culture medium, MicroSnap™ results do not constitute a guarantee of product quality.
6. Personnel must be trained in proper testing techniques and standard microbiological practices.

Hygiena Liability:

As with any culture medium, MicroSnap EB results do not constitute a guarantee of quality of food, beverage products or processes that are tested with these Devices. Hygiena will not be liable to user or others for any loss or damage, whether direct or indirect, incidental or consequential from use of this Device. If this product is proven to be defective, Hygiena's sole obligation will be to replace product, or at its discretion, refund the purchase price. Promptly notify Hygiena within 5 days of discovery of any suspected defect and return product to Hygiena. Please call Customer Service for a Returned Goods Authorization Number.

Contact Information:

If more information is required, please visit us at www.hygiena.com or contact us at:

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Step: 1 Incubation

1 **Surface**
10cm
10cm
1-i. **Surface:** Swab a 10x10cm area with Incubation Device.

OR

1-ii. **Liquids:** Add 1mL liquid food, beverage, or water sample directly to Incubation Device.

OR

1-iii. **Solid Samples:** Add 1mL of appropriate dilution of solid samples directly to Incubation Device.

2
2. Re-insert Snap-Valve bulb into swab tube.

3
3. Activate Device. Bend bulb, breaking Snap-Valve.

4
4. Lift bulb up (about 1 – 2 inches) and squeeze bulb to release liquid into bottom of tube. Replace bulb on to tube. Liquid should now be in bottom of tube.

5
5. Shake tube gently to mix sample in liquid.

6
6. Incubate at 37 ± 0.5 °C for 6 - 8 hours. Proceed to Step 2.

Step: 2 Detection

1
1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.

2
2. Transfer sample from Incubation Device to Detection Device.

3
3. Activate Detection Device by breaking Snap-Valve. Squeeze bulb to release liquid into tube. Liquid should now be in bottom of tube.

4
4. Shake tube gently to mix sample in liquid.

5a
5a. For EnSURE™ Touch, skip to 5b. On EnSURE™, insert Detection Device and press "OK" to initiate measurement.

6a
6a. Record RLU results and refer to the Results Interpretation Table for the conversion.

5b
5b. On EnSURE™ Touch, open the MicroSnap™ application and select "Quick Test" if you are testing a surface and have not programmed your sample or select Samples if you have programmed your sample. Press "Run Test."

6b
6b. EnSURE Touch automatically saves your results. To get the most value out of your EnSURE™ Touch, register and sync it wirelessly to SureTrend™ Cloud where you can generate meaningful reports and view datasets. Find out more at www.hygiena.com/suretrendcloud.