MicroSnap® EB (*Enterobacteriaceae*)

For using:

Enrichment options

- Product No. MS1-EB (MicroSnap® EB Enrichment Device)
- Product No. MS1-EB-BROTH-9ML (MicroSnap Enhanced EB Broth in 9 mL Vial)

Detection

- Product No. MS2-EB (MicroSnap® EB Detection Device)

Introduction

Description and Intended Use

MicroSnap® EB (*Enterobacteriaceae*) is a rapid bioluminogenic test for the detection and enumeration of *Enterobacteriaceae* bacteria in a sample and provides results in 6 to 8 hours. MicroSnap EB consists of an Enrichment Device containing proprietary growth media and a Detection Device containing bioluminogenic reagents in which biomarkers produced by bacteria are measured using a hand-held luminometer from Hygiena®.

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 greater the output of light. 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.

Some matrices, such as opaque liquid suspensions or samples with extreme pH, may require dilution (see Important Tips section). In these cases, we offer 9 mL vials containing the proprietary MicroSnap Enhanced EB Broth for use instead of the enrichment device.

Intended User

Laboratory personnel trained in standard microbiological practices are qualified to use MicroSnap EB devices.

Applicability

MicroSnap EB can be used for the enumeration of *Enterobacteriaceae* from environmental surfaces, product samples, water and other filterable liquids. If desired, MicrSnap EB devices can also provide qualitative results (absence/presence) for the same matrices.

Required Materials (Not Provided)

- EnSURE® Touch luminometer (Product No. ETOUCH)
- Dry Block Incubator (at 37 ± 0.5 °C) (Product No. INCUBATOR or INCUBATOR2)
- Block options for incubators:
  - 35-wells for swabs for INCUBATOR2 (Product No. IB001)
  - 15-wells for 9 mL vials for INCUBATOR2 (Product No. IB002)
  - 12-wells for swabs for INCUBATOR (Product No. IB003)
  - 6-wells for 9 mL vials for INCUBATOR (Product No. IB004)

Required Materials When Testing Product Samples (Not Provided)

- Sample bags
- Homogenizing equipment
- Pipettor and tips for 1 mL
- Product sample diluent options:
  - Buffered peptone water
  - Maximum recovery diluent
  - Butterfield's diluent
  - Sterile water

Important Tips Before Starting the Test

- For samples that may require dilution (e.g., opaque solutions; samples that may contain sanitizers, surfactants or other inhibitory compounds), use the MicroSnap Enhanced EB Broth for enrichment (for details, see Appendix and diagrams).
- Product samples can be stored prior to use at 2 to 8 °C for up to 2 days but must be equilibrated to room temperature (20 to 25 °C) before testing samples with MicroSnap EB.
- MicroSnap EB Enrichment Devices, MicroSnap Enhanced EB Broth Vials and MicroSnap EB Detection Devices must be equilibrated to room temperature before use.
- Use aseptic techniques: when collecting samples or transferring enriched samples, do not touch the swab or the inside of the enrichment device or vial with your fingers.

Test Procedure

Step 1: Enrichment

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

1. Collect and prepare the sample, according to sample type as noted:
    a. Surface Samples—Use the pre-moistened Enrichment Device to sample a 10 x 10 cm (4 x 4 inch) square area.

        Important swabbing technique tips:

        i. Apply sufficient pressure to create flex in the swab shaft.

        ii. Swab in a crisscross pattern vertically, horizontally and diagonally in both directions.

        iii. Rotate the swab while collecting the sample to maximize sample collection on the swab tip.

        iv. For irregular surfaces, ensure the swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample.

    b. Liquid Samples—Transfer 1 mL of a liquid or water sample directly to the Enrichment Device.

    c. Solid Product Samples—Transfer 1 mL of an appropriate suspension, e.g., 10% w/v food homogenate, directly to the Enrichment Device.

        i. Food homogenate should be prepared by weighing 10 or 50 g of food matrix and adding it to a stomacher bag containing 90 mL or 450 mL of diluent, respectively.

        ii. For unknown sample contamination, prepare and test 1:10 serial dilutions (i.e., 10%, 1% and 0.1%).

        iii. If replicate samples are required, then another 10 g or 50 g should be removed from the bulk matrix and the dilution series repeated. Replication can be achieved by drawing multiple 1 mL aliquots from either the 10%, 1% or 0.1% dilutions depending on the RLUs achieved.

        Note: When performing comparison testing, sample assays must be started within 10 minutes of each other for comparable results between methods.

    d. Re-attach the swab to the swab tube. The device should look the same as it did when first removed from the bag.

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

3. Separate the bulb and swab tube until the swab tip is above the fluid and squeeze the bulb to flush all the media into the swab tube. Ensure most of the broth is at the bottom of the swab tube.

4. Re-attach the swab to the swab tube firmly to seal the device and shake the tube gently to mix the sample and broth.

5. Incubate at 37 ± 0.5 °C for 6 hours ± 10 minutes or 7 hours ± 10 minutes for quantitative results (enumeration) or 8– 24 hours for qualitative results (absence/presence) (see Table 1).

Step 2: Detection

The detection procedure is described below and is also shown in diagrams (MicroSnap Enrichment Device or MicroSnap Enhanced EB Broth Vial).

Before beginning Step 2, turn on the luminometer. If you have programmed your MicroSnap sample in the luminometer, open the test screen of the sample you want to test.

Remember to equilibrate the MicroSnap EB Detection Device to room temperature (10 minutes at 20 to 25 °C) before use.

1. Shake the test device by either tapping on the palm of your hand 5 times or forcefully flicking in a downward motion once.

   Note: This is necessary to bring the liquid to the bottom of the tube, which will facilitate mixing of the enriched sample with the extractant in the tube.

2. Aseptically transfer 0.1 mL (2 drops) of enriched sample to the Detection Device.

   a. For MicroSnap Enrichment Devices, use the built-in dropper tip as a pipette:

      i. Squeeze and release the Enrichment Device bulb to mix and draw the sample into the bulb.

      ii. Aseptically open the Enrichment Device and the Detection Device by twisting and pulling to remove the bulbs.

      iii. Insert the Enrichment Device swab tip 3 cm (1 inch) into the top of the Detection Device tube and gently squeeze the Enrichment Device bulb to transfer 2 drops of the enriched sample into the tube.

      Note: A fill line is added to the tube as a reference. Inconsistent transfer volumes increase the variation of the test results.

   b. For MicroSnap broth vials:

      i. Remove the Enhanced Broth vial from the incubator then shake or vortex for 10 seconds to disperse the sample.

      ii. Aseptically uncap the vial and open the Detection Device by twisting and pulling to remove the bulbs.

      iii. Aseptically pipette 0.1 mL of the enriched sample directly into the Detection Device tube.

   c. Reassemble the Enrichment Device to its original state or recap the vial and return the sample to the incubator for potential retesting.

      Note: When testing replicates from the same enriched sample, all replicates must be performed within 10 minutes of each other to obtain comparable results.

3. Activate the Detection Device by holding the tube firmly and using your thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward. Squeeze the bulb 3 times to release all the liquid to the bottom of the tube.

4. Shake gently for 2 seconds to mix.

5. Immediately insert the whole device into the luminometer, close the lid and while holding unit upright, press the button to initiate the measurement.

6. EnSURE Touch luminometers display results in CFUs in 10 seconds.

   Note: MicroSnap samples can be programed directly on the luminometer or by using SureTrend® software.

Additional Information

Potential Limit of Detection

The limit of detection is the lowest level of viable aerobic bacteria that can be detected above a food matrix background when the assay is performed correctly and efficiently.

Table 1. Potential Dynamic Range (Limit of Detection) for the EnSURE Touch Luminometer.

Sample Type	CFU Range*		CFU Presence or Absence (Enrichment: 8 h ± 10 min)†
	Enrichment: 6 h ± 10 min	Enrichment: 7 h ± 10 min	
Surface	10 – 200,000 CFU/swab	10 – 10,000 CFU/swab	0 (absence) 1 CFU (caution) ≥2 CFU (presence)
Liquid (1 mL)	10 – 350,000 CFU/mL	10 – 10,000 CFU/mL	
Suspension of solid (10% w/v)	100 – 3,500,000 CFU/g	100 – 50,000 CFU/g	

* Additional factors, such as dilutions, incubation times and matrix types, can alter the ranges shown in Table 1. If sample contamination is above the ranges detailed in Table 1, then dilutions should be made so that the contamination is within the detectable range of the luminometer. For example:

- 1% suspension will be 1,000 – 500,000 CFU for a 7-hour incubation.
- 0.1% suspension will be 10,000 – 5,000,000 CFU for a 7-hour incubation.

† Incubation for presence/absence results can be extended up to 24 hours.

Interpretation of Results

Results on EnSURE Touch luminometers are shown in CFUs, providing qualitative (presence/absence) as well as quantitative (CFU/g or CFU/mL) results.

Where several dilutions are prepared and tested for samples with unknown contamination, the CFU/g or CFU/mL is calculated by multiplying the CFU result by the corresponding dilution factor. The EnSURE Touch luminometer software does this conversion, using the data generated from AOAC Validation Studies and internal testing.

Troubleshooting

Table 2 provides guidance on how to overcome some commonly seen sample effects. For additional protocol or matrix support, contact us at www.hygiena.com/support.

Table 2. Troubleshooting

Observation	Possible Cause	Recommended Action
Uncharacteristically high CFUs with some matrices, such as leafy greens	Some sample types naturally contain high levels of nucleotides that can increase CFU results.	Contact us for assistance with customizing the RLU-to-CFU conversion and the instrument threshold levels for your sample matrix.
Uncharacteristically low CFUs with thick, opaque or dark sample matrices, such as undiluted milk or chocolate	Interference with light detection by the luminometer can be caused by a blanching effect from the sample matrix.	Use the MicroSnap Enhanced EB Broth in 9 mL vials for enrichment. See Appendix for details.

Calibration and Controls

It is advisable to run positive and negative controls according to Good Laboratory Practice. Hygiena offers the following calibration verification products: CalCheck LED Calibration Verification Device (Product No. CAL).

Storage and Shelf Life

- Store at 2 to 8 °C (36 to 46 °F).
- Do not use past the expiration date on the label.

Disposal

Disinfect before disposal. MicroSnap devices can be disinfected by autoclaving or bleaching (soak unsealed devices 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 and Precautions

- MicroSnap device components do not pose any health risks 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).
- Do not use MicroSnap devices for diagnosis of conditions in humans and animals.
- As with any culture medium-based test, MicroSnap results do not constitute a guarantee of product quality.
- Avoid prolonged exposure to light.
- Devices and vials are designed for single use. Do not reuse.

Caution and User Responsibility

- MicroSnap devices have not been tested with all possible food products, food processes, testing protocols or with all possible microorganism strains.
- 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.
- The estimation of *Enterobacteriaceae* cannot be used as a proxy measurement for the presence or absence of *Salmonellae* species. For investigations of *Salmonellae* presence, a standard method *Salmonellae* test should be performed from food or environmental surfaces.
- Sampling should be done aseptically to avoid cross-contamination.
- It is the user's responsibility when selecting a test method to evaluate a sufficient number of samples.
- Verify proper incubation temperature and time for the test application.
- The incubation time will be 6 hours ± 10 minutes or 7 hours ± 10 minutes for quantitative results (enumeration) or 8 – 24 hours for qualitative results (presence/absence) as specified in the above instructions unless you have been directed otherwise by Hygiena's R&D team for custom applications that require different incubation times (or temperatures).
- Ensure proper sample dilution so that samples can be read within the luminometer's dynamic range.
- When testing multiple serial dilutions, all dilutions must be prepared and tested within 10 minutes of each other to obtain linear results.

- When testing replicates from the same enriched sample, all replicates must be performed within 10 minutes of each other to obtain comparable results.
- When performing comparison testing, sample assays must be started within 10 minutes of each other for comparable results between methods.

Hygiena Liability

As with any culture medium-based test, 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 the user or others for any loss or damage, whether direct or indirect, incidental or consequential, from use of these devices. If this product is proven to be defective, Hygiena's sole obligation will be to replace the product, or at its discretion, refund the purchase price. Promptly notify Hygiena within 5 days of discovery of any suspected defect and return the product to Hygiena; contact Customer Service for a Returned Goods Authorization Number.

Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

Appendix: Enrichment of Challenging Matrices with MicroSnap Enhanced EB Broth

MicroSnap Enhanced EB Broth contains 9 mL of a unique liquid medium designed to grow aerobic and facultative microorganisms while enhancing the production of biomarkers and specific enzymes diagnostic of *Enterobacteriaceae* and reducing sample interferences. The broth is primarily intended for applications requiring the detection of bacteria in challenging samples, such as opaque liquid suspensions.

MicroSnap Enhanced EB Broth is a ready-to-use media compatible with the following three detection devices: MicroSnap EB (MS2-EB), MicroSnap Coliform (MS2-COLIFORM) and MicroSnap *E. coli* (MS2-ECOLI) Detection Devices. Instructions in this insert are for enriching opaque solutions (e.g., milk) and other challenging food samples (e.g., spices) for detection of *Enterobacteriaceae*. For help developing a protocol for your matrix, including adjusting enrichment incubation temperatures, contact Hygiena for guidance.

Important Tips Before Starting the Test

- Visually inspect the liquid in the vial before use. The liquid should be clear and light straw color, not turbid or cloudy.
- Use a permanent marker to identify the sample on the vial label.

Step 1: Enrichment with MicroSnap Enhanced Nutrient Broth

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

1. Collect and prepare the sample using aseptic techniques:
   a. Liquid Samples—Add 1 mL of sample directly to the vial of Enhanced EB Broth.
   b. Solid Samples—Transfer 1 mL of a suitable sample dilution in sterile diluent directly to the vial of Enhanced EB Broth.
2. Replace and tighten the cap.
3. Shake or vortex for 10 seconds to mix contents.
4. Incubate the vial in a Hygiena Digital Dry Block Incubator at 37 ± 0.5 °C for 6 – 8 hours, depending on the sensitivity required (Table 3).

Table 3. Incubation Time and Potential Dynamic Range at 37 ± 0.5 °C.

Incubation Time*	CFU Range	Results
6 hours ± 10 minutes	500 – 250,000	Enumeration
7 hours ± 10 minutes	50 – 50,000	Enumeration
8 hours ± 10 minutes†	<5 – 5,000	Presence/absence

* Enumeration for Incubation periods outside of defined times have not been validated.

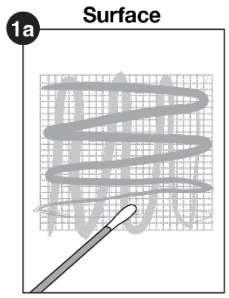
† Incubation for presence/absence results can be extended up to 24 hours.

Step 2: Detection

Follow instructions for detection as described above.

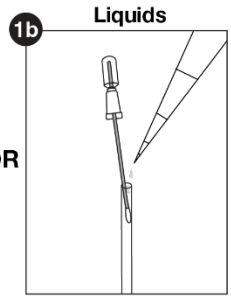
MicroSnap® EB Enrichment and Detection Devices

Step 1: Sample Enrichment



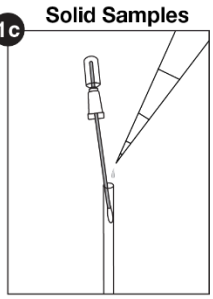
1a. Surface: Swab a 10 x 10 cm area with room-temperature* (RT) Enrichment Device.

OR

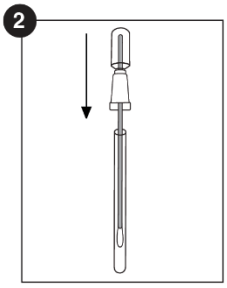


1b. Liquids: Add 1 mL of liquid food, beverage or water directly to RT Enrichment Device.

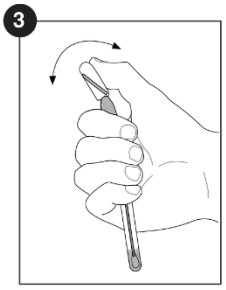
OR



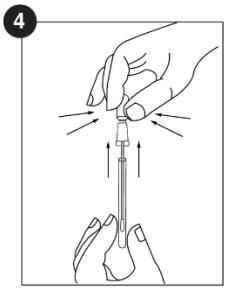
1c. Solid Samples: Add 1 mL of dilution of solid sample directly to RT Enrichment Device.



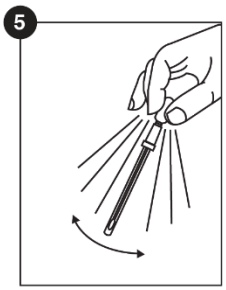
2. Re-insert Snap-Valve bulb into swab tube.



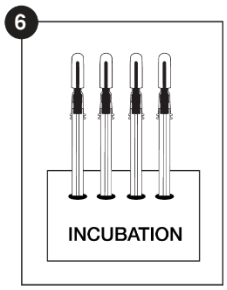
3. Activate Device. Bend bulb, breaking Snap-Valve.



4. Lift bulb up (1 – 2 inches) and squeeze to release liquid into bottom of tube.

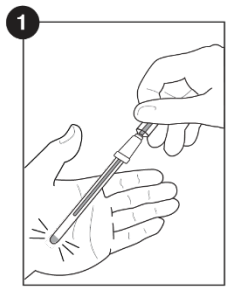


5. Replace bulb into tube and shake tube gently to mix sample in liquid.

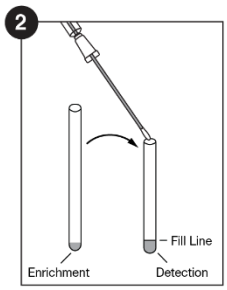


6. Incubate at 37 ± 0.5 °C for 6 or 7 h ± 10 min (quantitative) or 8 – 24 h (qualitative).

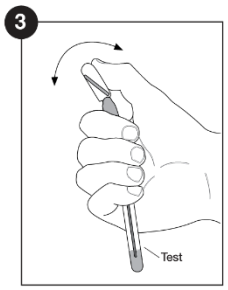
Step 2: Detection or Measurement



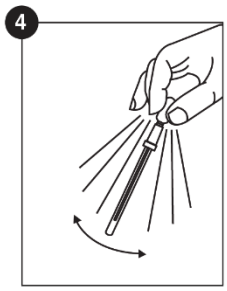
1. Equilibrate Detection Device to room temperature. Shake to bring liquid to the bottom.



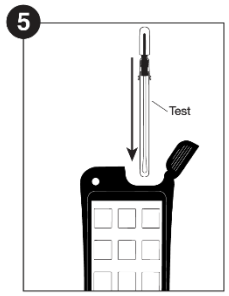
2. Aseptically transfer 2 drops (0.1 mL) of sample from Enrichment Device to Detection Device.



3. Activate Detection Device (Test) by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



4. Shake tube gently to mix sample in liquid.



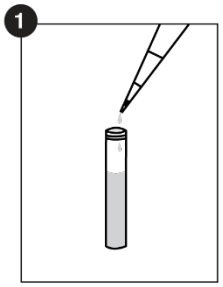
5. EnSURE® Touch, MicroSnap® application: If sample is programed, select sample; otherwise, select **Quick Test**. Then, press **Run Test**.

6. EnSURE Touch automatically saves results. Register and sync luminometer wirelessly to SureTrend® software to see reports and datasets.

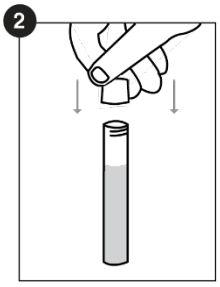
* Room temperature = 20 to 25 °C.

MicroSnap® Enhanced EB Broth Vial and MicroSnap Detection Device

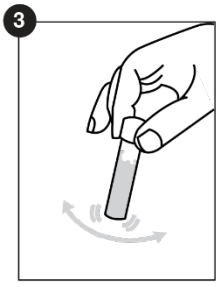
Step 1: Sample Enrichment



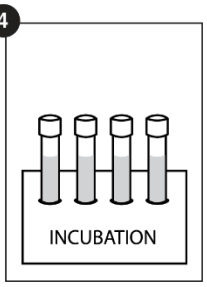
1. Equilibrate sample and EB broth at 20 to 25 °C. Add 1 mL of sample to Enhanced EB Broth.



2. Replace and tighten cap.

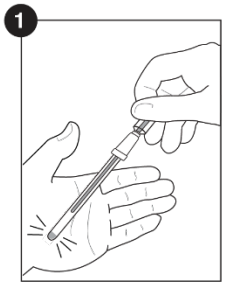


3. Shake or vortex for 10 seconds.

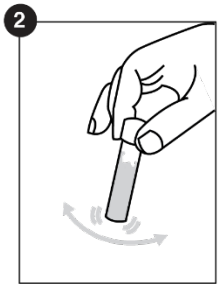


4. Incubate at 37 ± 0.5 °C for 6 or 7 h ± 10 min (quantitative) or 8 – 24 h (qualitative).

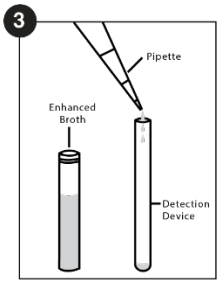
Step 2: Detection or Measurement



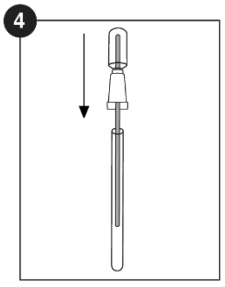
1. Equilibrate Detection Device to room temperature. Shake to bring liquid to bottom.



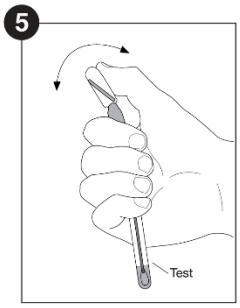
2. Shake or vortex for 10 seconds.



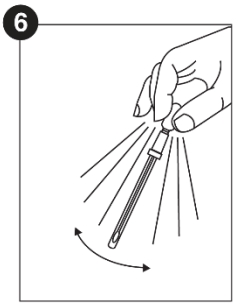
3. Aseptically transfer 0.1 mL of enriched sample to the Detection Device.



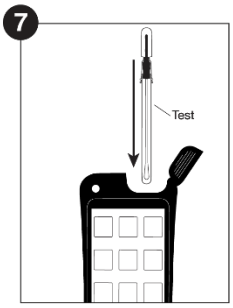
4. Reassemble Detection Device to original state.



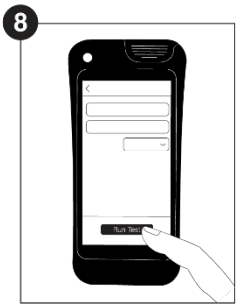
5. Activate device by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



6. Shake tube gently to mix sample in liquid.



7. Insert device into EnSURE® Touch. In the MicroSnap® application: If sample is programed, select sample; otherwise, select **Quick Test**. Then, press **Run Test**.



8. EnSURE Touch automatically saves results. Register and sync luminometer wirelessly to SureTrend® software to see reports and datasets.