

MicroSnap Enhanced EB Broth – 9ml

Directions for use with MicroSnap EB Detection Device (MS2-EB)

Matrix: Liquid Milk Products

Part No: MS1-EB-BROTH-9ML (100)

Description/Intended Use:

Hygiena's Enhanced EB Broth is a ready-to-use device compatible with MicroSnap EB (MS2-EB), MicroSnap Coliform (MS2-COLIFORM) and MicroSnap *E. coli* (MS2-ECOLI) detection devices.

Instructions in this insert are for MicroSnap EB (MS2-EB) Detection Devices with liquid milk samples only.

Enhanced EB Broth contains 9ml of unique liquid medium designed to grow aerobic and facultative microorganisms while enhancing production of biomarkers and specific enzymes diagnostic of Enterobacteriaceae, and reducing sample interferences. The broth has been primarily produced for liquid milk products. For use with other product samples or incubation times and temperatures other than $37 \pm 0.5^\circ\text{C}$, please contact Hygiena for guidance.

Required Materials (Not Provided):

- EnSURE luminometer (Part No. ENSURE)
- MicroSnap EB Detection Device (MS2-EB)
- Dry heating block incubator at $37 \pm 0.5^\circ\text{C}$
- Pipettes for transferring 1ml and 0.1ml volumes
- Vortex (optional)

Directions:

Instructional Video: www.youtube.com/HygienaTV

Step 1: Enrichment

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

Visually inspect liquid in tube prior to use. Liquid should be clear and light straw color, not turbid or cloudy. Use permanent marker to identify sample on tube label.

1. Allow milk sample and Enhanced EB Broth vial to equilibrate to room temperature (10 minutes at $22 - 26^\circ\text{C}$). Unscrew Enhanced EB Broth cap and aseptically add 1ml liquid milk sample.
2. Replace cap and tighten to secure.
3. Mix vial contents by hand-shaking or vortexing for 10 seconds. Incubate at $37 \pm 0.5^\circ\text{C}$ for 6 to 8 hours, depending on sensitivity required. Refer to Table 1 below.

Table 1: Incubation Time & Dynamic Range

Incubation Time	CFU Range	Results
6 hrs	500-250,000	Enumeration
7 hrs	50-50,000	Enumeration
8 hrs	<5-5000	Presence/Absence

Step 2: Detection

Detection procedure is described below and is also shown in Step 2 diagrams.

Turn on luminometer. If locations have been programmed, select location to be tested.

1. Allow MicroSnap EB Detection Device (Part # MS2-EB) to equilibrate to room temperature (10 minutes at $22 - 26^\circ\text{C}$). Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once. This will bring extractant liquid to bottom of tube.
2. Remove Enhanced EB Broth tube from incubator and mix by manually shaking or vortexing for 10 seconds to disperse sample.
3. Open Detection Device by twisting and pulling to remove bulb. Set aside. Using pipette, aseptically transfer 0.1ml of enriched sample directly into Detection Device tube.
4. Reassemble Detection Device to original state.
5. 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.

6. Shake gently to mix.
7. Immediately insert whole device into luminometer; close lid and holding unit upright, press "OK" button to initiate measurement. Results will appear after 15 second count down.
8. Result will be displayed in RLU (relative light units). Set RLU thresholds on instrument to correspond with required CFU limits. Refer to "Interpretation of Results" below for correlation.

Interpretation of Results:

Results are displayed as relative light units (RLU). RLU output is proportional to the starting inoculums and corresponding bacteria equivalent numbers (expressed as colony forming units, CFU). Table 2 shows equivalent colony forming unit (CFU) values to RLU. This will give an estimation of the Enterobacteriaceae CFU/mL from the original milk sample.

Table 2: Average relationship between CFU and MicroSnap EB RLU at 6, 7 and 8 hours incubation of 1mL of whole milk naturally contaminated with organisms at various levels (n=25 per level) incubated at $37 \pm 0.5^\circ\text{C}$

mean CFU/mL	EnSURE RLU		
	6 hours	7 hours	8 hours
<5	NA	NA	<5
10	NA	NA	<10
25	NA	NA	<25
50	NA	<10	<50
100	NA	<20	<100
250	NA	<50	<250
500	<10	<100	<500
1,000	<20	<200	<1,000
2,500	<50	<500	<2,500
5,000	<100	<1,000	<5,000
10,000	<200	<2,000	TNTC
25,000	<500	<5,000	TNTC
50,000	<1,000	TNTC	TNTC
250,000	<5,000	TNTC	TNTC
>250,000	TNTC	TNTC	TNTC

Salmonellae Investigations:

The estimation of Enterobacteriaceae cannot be used as a proxy measurement for presence or absence of Salmonellae species. For investigations of Salmonellae presence, a standard method Salmonellae test should be performed from food or from environmental surfaces.

Calibration & Controls:

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

- Calibration Control Kit for Hygiena luminometers (Part # PCD4000)
- MicroSnap Coliform & *E.coli* Positive Controls (Part # MS-PC-COLIFORM)

Storage & Shelf Life:

Store at $2 - 8^\circ\text{C}$. Devices have a shelf life of 12 months. 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.

MicroSnap Enhanced EB Broth – 9ml

Directions for use with MicroSnap EB Detection Device (MS2-EB)

Matrix: Liquid Milk Products

Part No: MS1-EB-BROTH-9ML (100)

Safety & Precautions:

MicroSnap Enhanced EB Broth does 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.

1. Enhanced EB Broth tubes are designed for a single use. Do not reuse.
2. Do not use devices after expiration date.
3. Sampling should be done aseptically to avoid cross contamination.
4. Avoid prolonged exposure to light.
5. Ensure proper dilution of sample to be read within the luminometer's dynamic range.
6. Ensure proper incubation temperature and time for the test application.
7. Do not use in the diagnosis of conditions in humans or animals.

User Responsibility:

MicroSnap Enhanced EB Broth does not contain any dyes, therefore, it alone will not detect bacteria colonies. The incubated broth will contain an active bacterial culture and should be used with MicroSnap detection devices. However, the incubated broth contains active viable organisms that can be subjected to further analysis by any other method. It is the user's responsibility to select a test method to evaluate a product by testing it with sufficient number of samples and microbial challenges to satisfy the user that the chosen method meets the user's criteria. Selection of broth, sampling method, testing protocol, preparation time and handling may influence recovery and enumeration of specific strains. MicroSnap is a time dependent test and it is important to follow instructions carefully to achieve optimal results.

Hygiena Liability:

As with any culture medium, MicroSnap Enhanced EB Broth 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:

Hygiena - Americas	Hygiena - International
Phone: 805.388.8007	Phone : +44 (0) 1923 818821
Fax: 805.388.5531	Fax: +44 (0) 1923 818825
Email: info@hygiena.com	Email: enquiries@hygiena.com

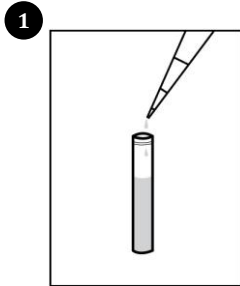
MicroSnap Enhanced EB Broth – 9ml

Directions for use with MicroSnap EB Detection Device (MS2-EB)

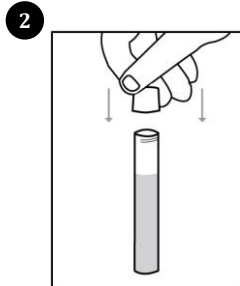
Matrix: Liquid Milk Products

Part No: MS1-EB-BROTH-9ML (100)

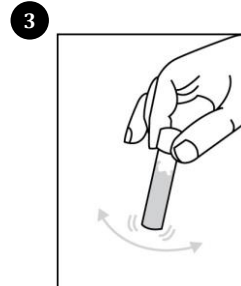
Step: 1 Enrichment of Milk Sample



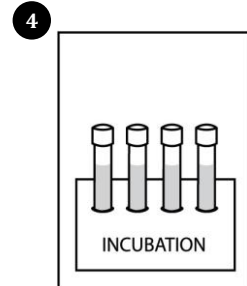
1. Allow milk sample and broth to equilibrate to 22 – 26 °C. Add 1mL milk sample directly to Enhanced EB Broth vial.



2. Replace cap and tighten to secure.

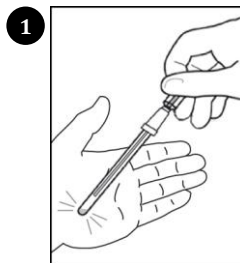


3. Shake by hand mixing or vortexing for 10 seconds.

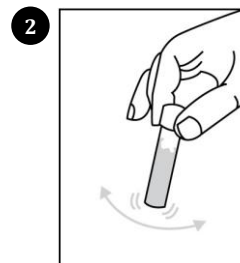


4. Incubate at 37 ± 0.5 °C for 6 - 8 hours. Refer to Table 1 for details.

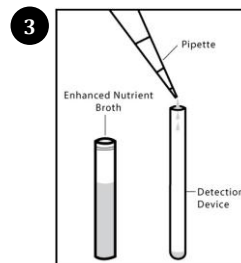
Step: 2 Detection / Measurement



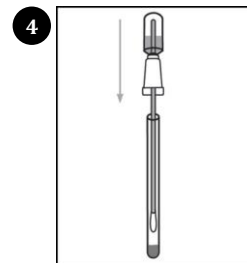
1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.



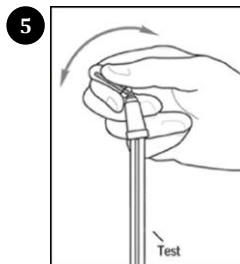
2. Shake by hand mixing or vortexing for 10 seconds.



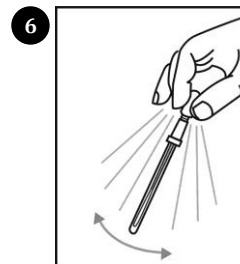
3. Aseptically transfer 0.1mL enriched sample from Enhanced EB Broth vial to Detection Device.



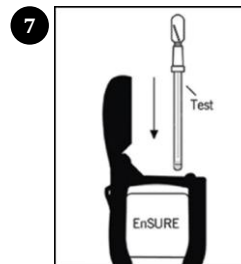
4. Reassemble device to original state.



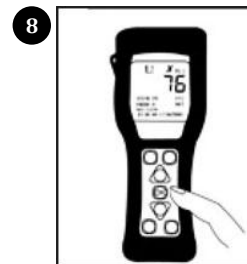
5. Activate device by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



6. After activation, shake tube gently to mix sample in liquid.



7. Insert device into EnSURE and press OK to initiate measurement.



8. Record results as RLU. Refer to Table 2 to interpret results.

Instructional Video:
www.youtube.com/HygienaTV