

MicroSnap™ Enhanced Nutrient Broth – 9ml

Directions for use with MicroSnap Total Detection Device (MS2-TOTAL)

Matrix: Solid and Liquid Products

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



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Description/ Intended Use:

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

Instructions in this insert are for MicroSnap Total (MS2-TOTAL)

Detection Devices with food samples.

Enhanced Nutrient Broth contains 9ml of unique liquid medium designed to grow aerobic and facultative microorganisms while enhancing production of biomarkers and specific enzymes diagnostic of Coliforms and *Escherichia coli*, and reducing sample interferences. The broth is primarily intended for applications requiring the detection of low numbers of bacteria in opaque liquid suspensions (e.g. 1 – 10 coliforms per ml of pasteurized milk). For help developing a protocol for your matrix, or for incubation temperatures other than 30 ± 0.5 °C using Enhanced Nutrient Broth and multiple Detection Devices, please contact Hygiena for guidance.

Required Materials (Not Provided):

- EnSURE™ luminometer (Part No. ENSURE)
- MicroSnap Total Detection Device (MS2-TOTAL)
- Hygiena™ Digital Dry Block Incubator at 30 ± 0.5 °C (Part No. INCUBATOR or INCUBATOR 2)
- Blocks for 9ml vials (IB002 or IB004)
- Pipettes for transferring 1ml and 0.1mL volumes
- Vortex (optional)

Directions:

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 sample and Enhanced Nutrient Broth vial to equilibrate to room temperature (10 mins at 22-26°C). For liquid samples, add 1ml directly to enhanced nutrient broth vial. For solid samples, make suitable dilution in sterile diluent, then add 1ml to enhanced nutrient vial.
2. Replace cap and tighten to secure.
3. Mix vial contents by hand-shaking or vortexing for 10 seconds.
4. Using a Hygiena Digital Dry Block Incubator, incubate at 30 ± 0.5 °C for 6 – 8 hours, depending on sensitivity required. Refer to Table 1 below.

Table 1: Incubation Time & Dynamic Range at 30 ± 0.5 °C

Incubation Time	CFU Range	Results	RLU to CFU Multiplier
6 hrs	500 – 250,000	Enumeration	50
7 hrs	50 – 25,000	Enumeration	5
8 hrs	<5 – 5,000	Enumeration	1

Note: Enumeration for Incubation periods longer than 8 hours cannot be guaranteed and has not been validated.

Incubation for extended periods of time e.g. overnight 18 - 24 hours or longer will yield presence/absence results only.

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 Total Detection Device (Part # MS2-TOTAL) to equilibrate to room temperature (10 minutes at 22 – 26 °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 Nutrient Broth tube from incubator and mix by hand-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. 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.
5. Shake gently to mix.
6. 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.
7. 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 inoculum and corresponding bacteria equivalent numbers (expressed as colony forming units, CFU). Using the RLU to CFU Multiplier stated in Table 1 will give an estimation of the Total CFU/mL from the original sample.

E.g. To convert an RLU result after a 7 hour incubation:

$$237 \text{ (RLU result)} \times 5 \text{ (7hour Multiplier)} = 1,185 \text{ CFU/ml}$$

A further example of RLU to CFU conversion is shown in Table 2.

At very high RLU values >5000 the system approaches the upper limit of quantification and results become less precise. The optimum RLU range is 5 to 5000RLU.

Correlation between RLU and CFU is matrix dependent and the specific RLU to CFU Multiplier used should be validated by customers for the specific matrices being tested.

Table 2: Example of relationship between CFU and MicroSnap Total RLU at 6, 7, and 8 hours incubation of 1mL of whole milk naturally contaminated with organisms at various levels

mean CFU/mL	EnSURE RLU		
	6 hours (Multiplier = 50)	7 hours (Multiplier = 5)	8 hours (Multiplier = 1)
<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

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

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 No. PCD4000 and CAL)

Storage & Shelf Life:

- Store at 2 – 8 °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.

Safety & Precautions:

MicroSnap Enhanced Nutrient 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 Nutrient Broth vials 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 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 Nutrient 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 Nutrient 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

Phone: 805.388.8007

Fax: 805.388.5531

Email: info@hygiena.com

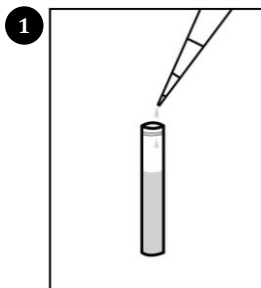
Hygiena - International

Phone : +44 (0) 1923 818821

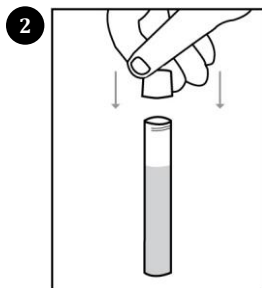
Fax: +44 (0) 1923 818825

Email: enquiries@hygiena.com

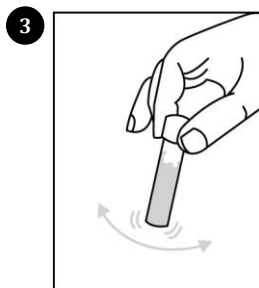
Step: 1 Enrichment of Sample



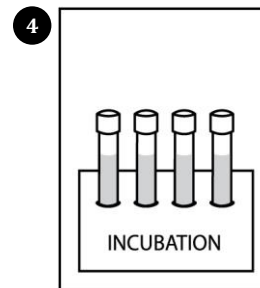
1. Allow sample and broth to equilibrate to 22 – 26 °C. Add 1mL of appropriate dilution of solid samples directly to Enhanced Nutrient Broth Vial.



2. Replace cap and tighten to secure.



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



4. Incubate at 30 ± 0.5 °C for 6 - 8 hours. Refer to Table 1 for details.

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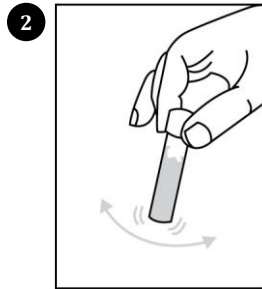
Matrix: Solid and Liquid Products

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

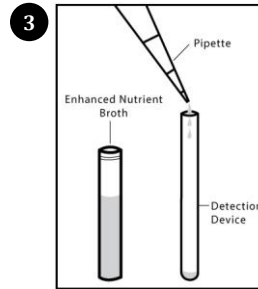
Step: 2 Detection / Measurement



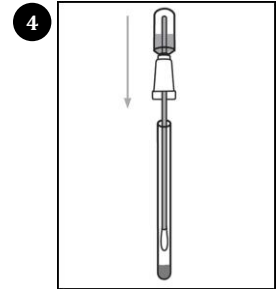
1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.



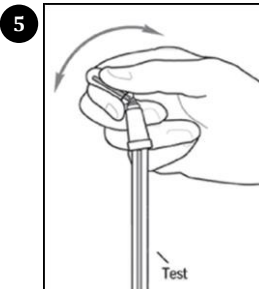
2. Shake by hand mixing or vortexing for 10 seconds.



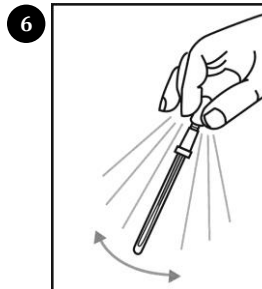
3. Aseptically transfer 0.1mL enriched sample from Enhanced Nutrient Broth tube to Detection Device.



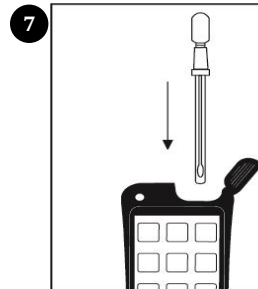
4. Reassemble device to original state.



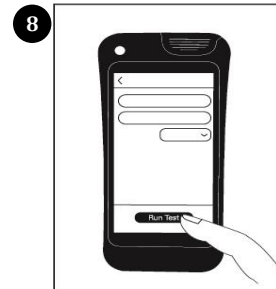
5. Activate device by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



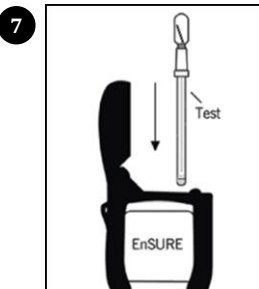
6. After activation, shake tube gently to mix sample in liquid.



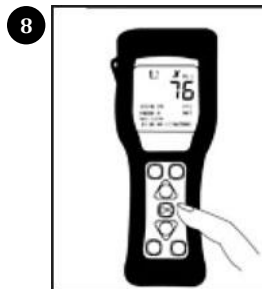
7. **EnSURE Touch:** Insert device into EnSURE Touch and press Run Test to initiate measurement.



8. **ENSURE Touch:** Results will be displayed on the screen.



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



8. **EnSURE:** Record results as RLU.