

Technical Data Sheet

Listeria Recovery and Enrichment Broth (LREB)

INTENDED USE AND APPLICATION

Listeria Recovery and Enrichment Broth (LREB) is a selective medium specifically optimized for single-step recovery and enrichment of *Listeria* species from food and environmental samples.

PRODUCT INFORMATION

SKU	DESCRIPTION	UOM	QUANTITY
DMR-LREB-1KG	<i>Listeria</i> Recovery and Enrichment Broth (LREB) Media	Canister	1 Canister (1 Kg)
DMR-LREB-1KG-CTN	<i>Listeria</i> Recovery and Enrichment Broth (LREB) Media	Case	6 Canisters (1 Kg/each)
DMR-LREB-20KG	<i>Listeria</i> Recovery and Enrichment Broth (LREB) Media	Bucket	1 Bucket (20 Kg)
ERM(ERMD)-LREB-0090-096	Enrichment Ready - LREB Media for 90 mL Enrichment	Case	96 Pouches
ERM(ERMD)-LREB-0225-096	Enrichment Ready - LREB Media for 225 mL Enrichments	Case	96 Pouches
ERM(ERMD)-LREB-1000-096	Enrichment Ready - LREB Media for 1.0 L Enrichments	Case	96 Pouches
ERM(ERMD)-LREB-1125-096	Enrichment Ready - LREB Media for 1.125 L Enrichments	Case	96 Pouches
ERM(ERMD)-LREB-3375-096	Enrichment Ready - LREB Media for 3.375 L Enrichments	Case	96 Pouches
RTM-LREB-0002-50	<i>Listeria</i> Recovery and Enrichment Broth (LREB) 2mL	Case	50 Pieces

Ready to use Media (RTM) help shorten preparation times, reduce contamination risks, and save money. Enrichment Ready Media is produced in foil (ERM) or dissolvable (ERMD) pouches to help streamline culture media preparation. LREB is weighed into individual ready-to-use pouches and sterilized which saves the end user time. LREB pouches are ready to hydrate in sterile water, eliminating the need for an autoclave in media preparation steps.

PRINCIPLE

Listeria Recovery and Enrichment Broth (LREB) combines nutritional components with additional ingredients that are necessary to selectively improve recovery and growth of *Listeria*. The selective agents present in LREB have been optimized to efficiently inhibit competing normal bacterial flora without affecting the growth of *Listeria* species. LREB is formulated for buffering capacity to ensure growth in a variety of matrices.

FORMULA

COMPONENT	QUANTITY	UNITS
Nutritive Mix	28.5	g
Sodium Chloride	2.5	g
Recovery Agents	1	g
Selective Agents	5	g
Water	1	L

Final pH: 7.2 ± 0.2 at 25°C

APPEARANCE

Dehydrated: Powder is homogeneous, free flowing, and beige.

Prepared: Autoclaved prepared broth is a translucent dark beige color. A light precipitate may occur during storage that returns into solution with a slight mix. Enrichment Ready Media broth is a beige color.

PRECAUTIONS

This product is for In Vitro diagnostic use only. Do not ingest, inhale, or allow to come into contact with skin. Observe approved biohazard precautions and aseptic techniques. Biosafety level 2 procedures should be exercised (BMBL, <http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf> or current site). Extreme care should be taken in handling test samples and enrichment broths. All inoculated enrichment broths may contain various pathogens whether or not they contain *Listeria* species. The medium is to be used only by adequately trained and qualified laboratory personnel in a laboratory setting. All laboratory specimens should be considered infectious and handled accordingly.

ADDITIONAL MATERIALS REQUIRED

ALL SAMPLES

- Autoclave
- Distilled / deionized water
- Sterile stomacher / blender bags or equivalent with and without filter
- Stomacher / blender or equivalent
- SIMUL-qPCR *Listeria* species and *monocytogenes* Assay Kit (SKU#: SMRT-LSLM-096)
- Incubator: at $30 \pm 1^\circ\text{C}$
- Incubator: at $35 \pm 1^\circ\text{C}$
- Routine laboratory equipment

ENVIRONMENTAL SAMPLES

- Quick Release Sampler Hydrated with Neutralizing and Recovery Buffer (SKU#: SC-SQRS-NRB-100)
- Quick Release Sampler Hydrated with Neutralizing Buffer (SKU#: SC-SQRS-NB-100)
- Quick Release Sampler Hydrated with Lethen Broth (SKU#: SC-SQRS-LB-100)
- Quick Release Sampler Hydrated with Buffered Peptone Water (SKU#: SC-SQRS-BPW-100)
- Quick Release Sampler Hydrated with DE-Neutralizing Broth (SKU#: SC-SQRS-DENB-100)
- AFD MEMP Swab Kit (SKU#: MEMP-SWB-032)
- Other commercially available swabs

PROCEDURE

MEDIA PREPARATION - AUTOCLAVE METHOD

1. Use a clean bottle for each liter of medium preparation.
2. Shake container of dry enrichment medium before each use.
3. Measure 37.0 g of powder into the bottle and add 1 L of distilled water.
4. Constantly stir and heat solution until powder is dissolved. The acceptable pH is 7.2 ± 0.2 .
5. Sterilize the bottle(s) of prepared medium by autoclaving at 121°C for 15 min.
6. Cool bottle(s) to room temperature. Media is stable at room temperature or can be stored at $2-8^\circ\text{C}$ for up to 45 days. Keep away from light.

MEDIA PREPARATION - NON-AUTOCLAVE METHOD

1. Prepare a sterile and clean bottle for each liter of medium preparation.
2. Shake container of dry enrichment medium before each use.
3. Measure 37.0 g of powder into the bottle and add to 1 L of sterile distilled or deionized water.
4. Constantly stir and heat solution until powder is dissolved. The acceptable pH is 7.2 ± 0.2 .
5. Cool the prepared medium to the appropriate temperature ($35 \pm 1^\circ\text{C}$) and use immediately.

MEDIA PREPARATION – ENRICHMENT READY MEDIA

1. Prepare a sample bag with pre-warmed ($35 \pm 1^\circ\text{C}$) laboratory grade ASTM D1193 Type 2 water with the volume respective of the pouch size.
2. If using a foil pouch, tap pouch to compact media away from the tear mark. Pinch and open the perforated tear mark and empty the contents into the sample bag. If using a dissolvable pouch, place pouch into in prepared bag from Step 1. Refer to Enrichment of Samples tables for acceptable media volumes.
3. Shake, stomach or hand mix until powder is dissolved.
4. Use within three to four hours while maintaining pre-warmed conditions ($35 \pm 1^\circ\text{C}$).

ENVIRONMENTAL SURFACE SAMPLE PREPARATION

1. When ready to test, pre-warm the prepared LREB to $35 \pm 1^\circ\text{C}$.
2. Add the pre-warmed LREB to each sponge sample in its sample bag. Refer to Enrichment of Samples tables for acceptable media volumes.
3. Homogenize the sample for 30 seconds in a stomacher / blender or equivalent. Hand mixing is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed bag for approximately one minute.
4. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

FOOD SAMPLE PREPARATION

1. Aseptically sample the product and place in a sterile bag.
2. When ready to test, pre-warm the prepared LREB to $35 \pm 1^\circ\text{C}$.
3. Add the pre-warmed LREB to each sample. Refer to the Enrichment of Samples tables for acceptable media volumes.
4. Homogenize the sample for 30 seconds in a stomacher / blender or equivalent. Hand mixing is an acceptable alternative for stomaching. To hand mix, massage each sponge that is in the sealed bag for approximately one minute.
5. Incubate the sample. Refer to Enrichment of Samples tables for enrichment conditions.

LREB ENRICHMENT OF SAMPLES PROTOCOL

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Environmental Swab*	/Swab	9 mL \pm 1 mL
AFD MEMP Reserve Swab*	/Swab	9 mL \pm 1 mL
Environmental Sponge*	/Sponge	90 mL \pm 10 mL
Frankfurter	/125 g	1 L \pm 50 mL
RTE Sliced Turkey	/125 g	1 L \pm 50 mL
Soft Fresh Cheese (Raw)	/25 g	225 mL \pm 15 mL
Chicken Salad	/25 g	225 mL \pm 15 mL
Ice Cream	/25g	225 mL \pm 15 mL
Cooked Eggs	/25 g	225 mL \pm 15 mL
Pasteurized Milk	/25 g	225 mL \pm 15 mL
Frozen/Cooked Shrimp	/25 g	225 mL \pm 15 mL

*Validated surfaces are stainless steel, plastic, rubber, ceramic tile, and sealed concrete

Enrichment Incubation: $30 \pm 1^\circ\text{C}$ for 30-36 hours

ANALYSIS OF ENRICHED SAMPLES

At the end of the enrichment phase, proceed to the appropriate protocol. See Product Information Sheets and/or Kit Inserts for Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Listeria* species and *monocytogenes* Assay and/or Chrom-Assured *Listeria* Detection Plates.

PRODUCT STORAGE AND EXPIRATION

For dehydrated media, store either pouches or sealed bottles containing the powder at 2 - 30°C. Once opened, protect from moisture and light by keeping container tightly closed. For ready to use media, store at 2 - 8°C

For all product types, the expiry date is indicated on the package. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original beige color. Expiry applies to medium in its intact container when stored as directed.

DISPOSAL

Dispose of all materials used and the enrichment medium by autoclaving or according to approved practices. Ensure that all biohazard waste is disposed of according to local, municipal, provincial, state and/or federal regulations.

QUALITY CONTROL

All products manufactured by Applied Food Diagnostics, Inc. are incorporated into a quality assurance program from the time the raw materials arrive in the factory through to marketing the end product. Each batch of end product undergoes quality control, and is only marketed if it complies with acceptance criteria. Documentation concerning production and verification of each batch is archived. A Certificate of Analysis of this quality control and Safety Data Sheets are available on the web at www.appliedfooddiagnostics.com.

APPEARANCE

Dehydrated: Powder is homogeneous, free flowing, and beige.

Prepared: Autoclaved prepared broth is translucent dark beige color. A light precipitate may occur during storage that returns into solution with a slight mix. Enrichment Ready Media broth is a beige color.

CULTURAL RESPONSE (PRODUCTIVITY AND SELECTIVITY TESTING)

The medium was prepared according to label directions and 10mL volumes were inoculated with the organisms listed below. Culture broths were incubated at 30 ± 1°C under an aerobic atmosphere and examined for growth at 24 hours. Following incubation, each culture was examined for turbidity and the SIMUL-qPCR assay was performed to determine growth promotion. Also, 1-10µL of each culture was streaked onto a Chrom-Assured *Listeria* Detection Plate, incubated at 37°C ± 1°C, and examined for growth at 22-26 hours. The table below outlines the results.

MICROORGANISM	ATCC/CATALOG NUMBER	APPROX. INOCULUM (CFU/mL)	EXPECTED RESULTS		
			Growth in LREB	Reaction on Chrom-Assured <i>Listeria</i> Detection Plate	Result on <i>Listeria</i> SIMUL-qPCR
<i>Listeria monocytogenes</i> 4b	B-33000	10-300	Good Growth	Blue colonies	Positive
<i>Listeria monocytogenes</i> 1/2b	B-33258	10-300	Good Growth	Blue colonies	Positive
<i>Listeria innocua</i>	B-33003	10-300	Good Growth	Blue colonies	Positive
<i>Listeria ivanovii</i>	B-33017	10-300	Good Growth	Blue colonies	Positive
<i>E. coli</i> O157:H7	ATCC 35150	10-300	Inhibition	No colonies	Negative
<i>Salmonella enteritidis</i>	SGSC 2475	10-300	Inhibition	No colonies	Negative
<i>Staphylococcus aureus</i>	NR-46412	10-300	Inhibition	No colonies	Negative
<i>Citrobacter freundii</i>	ATCC 43864	10-300	Inhibition	No colonies	Negative

TECHNICAL INFORMATION



If you have any questions or experience issues with this product, please contact our support staff via email (support@appliedfooddiagnostics.com). For more information about Applied Food Diagnostics, Inc., please visit us at our website (www.appliedfooddiagnostics.com).

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