

## TECHNICAL DATA SHEET

### ENTEROHEMORRHAGIC *E. coli* RECOVERY AND ENRICHMENT BROTH (EREB)

#### INTENDED USE AND APPLICATION

Enterohemorrhagic *E. coli* Recovery and Enrichment Broth (EREB) is a selective medium specifically optimized for single-step recovery and enrichment of Enterohemorrhagic *E. coli* (EHEC) from raw beef trim and ground beef. The medium can also be used for the recovery and enrichment of *Salmonella* as a single-enrichment method alongside EHEC in beef samples.

#### PRODUCT INFORMATION

SKU	DESCRIPTION	UOM	QUANTITY
DMR-EREB-1KG	EHEC Recovery and Enrichment Broth (EREB) Media	Canister	1 Canister (1 Kg)
DMR-EREB-1KG-CTN	EHEC Recovery and Enrichment Broth (EREB) Media	Case	6 Canisters (1 Kg each)
DMR-EREB-20KG	EHEC Recovery and Enrichment Broth (EREB) Media	Bucket	1 Bucket (20 Kg)
ERM(ERMD)-EREB-0090-096	Enrichment Ready - EREB Media for 90 mL Enrichments	Case	96 Pouches
ERM(ERMD)-EREB-0225-096	Enrichment Ready - EREB Media for 225 mL Enrichments	Case	96 Pouches
ERM(ERMD)-EREB-1000-096	Enrichment Ready - EREB Media for 1.0 L Enrichments	Case	96 Pouches
ERM(ERMD)-EREB-3375-096	Enrichment Ready - EREB Media for 3.375 L Enrichments	Case	96 Pouches
RTM-EREB-0002-50	<i>E.coli</i> Recovery Enrichment Broth (EREB) 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. EREB is weighed into individual ready-to-use pouches and sterilized which saves the end user time. EREB pouches are ready to hydrate in sterile water, eliminating the need for an autoclave in media preparation steps.

#### PRINCIPLE

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

#### FORMULA

COMPONENT	QUANTITY	UNITS
Nutritive Mix	27.5	g
Sodium Chloride	5.0	g
Buffering Phosphates	4.0	g
Selective Agents	1	g
Recovery Agents	0.25	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 translucent and dark beige to amber. 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 EHEC or *Salmonella* 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 Top 7 STEC Assay Kit (SKU#: SMRT-T7-096)
- SIMUL-qPCR *Salmonella* Assay Kit (SKU#: SMRT-SAL-096)
- Chrom-Assured *Salmonella* Detection Plates (SKU#: CDP-SAL-032)
- Incubator: at  $42 \pm 1^\circ\text{C}$
- Incubator: at  $45 \pm 1^\circ\text{C}$
- Routine laboratory equipment

## 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 37.8 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  $7.2 \pm 0.2$ .
5. Sterilize the bottle(s) of prepared medium by autoclaving at  $121^\circ\text{C}$  for 15 min.
6. Cool bottle(s) to room temperature. Media is stable at room temperature, or it 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 37.8 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  $7.2 \pm 0.2$ .
5. Cool the prepared medium to the appropriate temperature ( $45 \pm 1^\circ\text{C}$ ) and use immediately.

### MEDIA PREPARATION – ENRICHMENT READY MEDIA

1. Prepare a sample bag with pre-warmed ( $45 \pm 1^\circ\text{C}$ ) 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 ( $45 \pm 1^\circ\text{C}$ ).

## FOOD SAMPLE PREPARATION

1. Aseptically sample the product and place in a sterile bag.
2. When ready to test, pre-warm the prepared EREB to  $45 \pm 1^\circ\text{C}$ .
3. Add the pre-warmed EREB to each sample. Refer to 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.

## EREB ENRICHMENT OF SINGLE SAMPLES USING AFD SIMUL-qPCR TOP 7 STEC PROTOCOL

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Raw Ground Beef	/375 g	1 L $\pm$ 50 mL
Raw Beef Excision Sample N60	/375 g	1 L $\pm$ 50 mL

Enrichment Incubation:  $42 \pm 1^\circ\text{C}$  for 10-18 hours.

## EREB ENRICHMENT OF POOLED SAMPLES USING AFD SIMUL-qPCR TOP 7 STEC PROTOCOL

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Raw Ground Beef	/375 g	1 L $\pm$ 50 mL
Raw Beef Excision Sample N60	/375 g	1 L $\pm$ 50 mL

Enrichment Incubation:  $42 \pm 1^\circ\text{C}$  for 12-18 hours.

## EREB ENRICHMENT OF SINGLE SAMPLES USING AFD SIMUL-qPCR SALMONELLA PROTOCOL

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Raw Ground Beef	/375 g	1 L $\pm$ 50 mL
Raw Beef Excision Sample N60	/375 g	1 L $\pm$ 50 mL

Enrichment Incubation:  $42 \pm 1^\circ\text{C}$  for 10-18 hours.

## EREB ENRICHMENT OF POOLED SAMPLES USING AFD SIMUL-qPCR SALMONELLA PROTOCOL

MATRIX	SAMPLE SIZE / ANALYSIS UNIT	MEDIA VOLUME
Raw Ground Beef	/375 g	1 L $\pm$ 50 mL
Raw Beef Excision Sample N60	/375 g	1 L $\pm$ 50 mL

Enrichment Incubation:  $42 \pm 1^\circ\text{C}$  for 12-18 hours.

## ANALYSIS OF ENRICHED SAMPLES

At the end of the enrichment phase, proceed to the appropriate protocol. See AFD Product Information Sheets and/or Kit Inserts for Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) Top 7 STEC Assay, Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Salmonella* Assay, and/or Chrom-Assured *Salmonella* Detection Plates.

## PRODUCT STORAGE AND EXPIRATION

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

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](http://www.appliedfooddiagnostics.com).

### APPEARANCE

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

Prepared: Autoclaved prepared broth is translucent and dark beige to amber. 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 10 mL volumes were inoculated with the organisms listed below. Culture broths were incubated at  $42 \pm 1^\circ\text{C}$  under 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, 1-10  $\mu\text{L}$  of each culture was streaked onto a Chrom-Assured *Salmonella* Detection Plate, incubated at  $37^\circ\text{C} \pm 1^\circ\text{C}$ , and examined for growth at 22-26 hours. The table below outlines the results.

MICROORGANISM	APPROX. INOCULUM (CFU/mL)	EXPECTED RESULTS		
		Growth in EREB	Reaction on Chrom-Assured <i>Salmonella</i> Detection Plate	Result on SIMUL-qPCR
<i>Escherichia coli</i> O157:H7	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O103	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O26	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O145	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O111	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O45	10-300	Good Growth	Blue Colonies	Positive
<i>Escherichia coli</i> O121	10-300	Good Growth	Blue Colonies	Positive
<i>Salmonella enteritidis</i>	10-300	Good Growth	Purple Colonies	Positive
<i>Escherichia coli</i>	10-300	Good Growth	Blue Colonies	Negative
<i>Escherichia fergusonii</i>	10-300	Good Growth	Blue Colonies	Negative
<i>Carnobacterium divergens</i>	10-300	Complete Inhibition	No Colonies	Negative
<i>Enterococcus faecalis</i>	10-300	Complete 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](mailto:support@appliedfooddiagnostics.com)). For more information about Applied Food Diagnostics, Inc., please visit us at our website ([www.appliedfooddiagnostics.com](http://www.appliedfooddiagnostics.com)).

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## **DISCLAIMER**

This product and its performance characteristics were developed by Applied Food Diagnostics, Inc., for laboratory use. Any deviations from this protocol are not authorized by Applied Food Diagnostics, Inc.

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