D/E NEUTRALIZING MEDIA

PRODUCTS:

Plated Media:[a]

D/E Neutralizing Agar, Irradiated	Barrier Wrap™ :	P8240 (100mm), P8035 (contact plate) P8035L (LockSure® contact plate)
	Triple Wrap:	P8050 (contact plate), P8050L (LockSure contact plate)
D/E Neutralizing Broth	B8132 (90 ml), T8200 (10 ml)	

[a]See catalog for ordering options

PURPOSE:

D/E (Dey-Engley) Neutralizing agar is used to cultivate a broad range of microorganisms while neutralizing disinfectants and antimicrobials.

PRINCIPLE:

D/E Neutralizing agar has the capability to neutralize a broad spectrum of disinfectants and antimicrobials which have inherent bacteriostatic properties. Viable bacteria may be held in bacteriostasis when affected by a strongly bacteriostatic substance, resulting in a false negative (no growth) test result. This media neutralizes disinfectant and antimicrobial compounds by the addition of sodium thioglycolate, sodium thiosulfate, sodium bisulfite, lecithin, and polysorbate 80. Sodium thiosulfate neutralizes iodine and chlorine. Sodium bisulfite neutralizes formaldehyde and gluteraldehyde. Sodium thioglycollate neutralizes mercurials. Lecithin neutralizes quaternary ammonium compounds and polysorbate 80 neutralizes phenols, hexachlorophene, formalin and when combined with lecithin, ethanol.

Casein peptones supply nutritious organic nitrogen, necessary for the growth of many organisms. Yeast extract provides B vitamins and proteins for supplementary nitrogen and carbon. Dextrose lends itself as a fermentable carbohydrate. The release of acids from fermentation lends to a colorimetric change in the bromcresol purple.

Gamma irradiation of the packaged media allows this product to be used in critical environments where introduction of contaminants are not desired. With Triple Wrap, aseptic removal of the inner packaging from the outer wrap allows the product to be moved into a clean room environment without the contaminated outer wrap. Product in the second or third inner wrap eliminates the need to aseptically rewrap the product for transport or storage and possibly contaminating the media. Barrier Wrap is a unique material that is impervious to pre-sterilizing agents such as vaporized hydrogen peroxide. Barrier Wrap is designed for isolator environmental testing. The LockSure locking plate secures the sample in case of mishaps by preventing the lid from separating and compromising the contents.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) D/E Neutralizing Agar*:

Pancreatic Digest of Casein	5.00 g
Yeast Extract	2.50
Dextrose	10.00
Sodium Thioglycollate	1.00
Sodium Thiosulfate	6.00
Sodium Bisulfite	2.50
Lecithin (Soybean)	7.00
Polysorbate 80	5.00
Bromcresol Purple	0.02
Agar	15.00

Final pH 7.9 ± 0.2 at 25°C

(2) D/E Neutralizing Broth

Same as **(1)** above except without the addition of agar

Final pH 7.6 ± 0.2 at 25°C

*Adjustments may be required to meet performance standards and to compensate for the effects of media irradiation.

PRECAUTIONS:*

For laboratory use only. Observe approved biohazard precautions.

Breatheable bags are used with triple-wrapped plates for maximum sterility assurance. However, fluctuation of temperature and/or humidity due to changes that occur during shipping and/or storage environment occasionally result in accumulation of moisture. If excessive moisture is observed, packages may be stored agar side up at room temperature (not exceeding 30°C) for up to 10 days without affecting the shelf life.

Storage: Upon receipt store at 2-8°C away from light. Media should not be used if there are signs of contamination, deterioration (i.e. shrinking, cracking, or discoloration), or if the expiration date has passed. Do not open outer wrapping until ready to use. Media can be inoculated up to the expiration date and incubated for the appropriate incubation period.

Limitations: D/E Neutralizing media are nonselective; for definitive identification of microorganisms, subculturing to plated media may be necessary as well as biochemical/serological testing. See appropriate references.

When the integrity of the seal or outer wrap is compromised, the product is no longer considered sterile.

Molds or spreading colonies can make accurate counting difficult. Unless a statistical method for monitoring is designed, the results can be uninterpretable or misleading.

The complexity of the surface (irregular, curved, porous, rough, or textured) to be tested may present sampling challenges for contact plates.

No single assay can characterize completely the microbial contamination in a specific area. A complete contamination control program should emphasize traffic control, special dress code procedures in critical areas, suitable ventilation, as well as good cleaning and disinfecting practices.

PROCEDURE:*

Specimen collection: Samples should be collected using appropriate techniques. Transportation of samples should be done in a timely manner using appropriate methods. See appropriate reference materials for more details.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Inoculate using standard reference guidelines.

The sealed outer wrapper of triple-wrapped plates may be opened aseptically by peeling apart the clear film from the white film at the edge or by cutting with sterile scissors. If sterility of the inner packaging is required, then appropriate procedures should be used to maintain the sterility of the inner contents.

Incubate media aerobically 1-3 days for bacteria at 32.5°C, and 1-5 days for yeasts and fungi at 22.5°C.

Interpretation:

Fermenting microorganisms such as *Escherichia coli* and *Salmonella* will change the color of the media from purple to yellow. All other non-fermentative facultative bacteria will grow without producing a color change in the media.

For plated media: Growth should be as isolated colonies, not growing confluently. Count isolated colonies and group them according to their phenotypic appearance. If spreading colonies must be counted, then use the following criteria to count as one colony.

- a spreader that developed a film of growth between the agar and the bottom of the petri dish
- a colony that spreads on a film of water at the edge or over the agar surface
- a chain of colonies that appears to be caused by disintegration of a bacterial clump; count each chain as a single colony; do not count each colony in each chain

For broth media: Visually examine the broth for turbidity. Subculture onto plated media to confirm growth using appropriate techniques. The media will turn yellow if fermenting microorganisms are present.
Colonies that require further identification should be subcultured for purity and then tested by biochemical, serological, and microscopic means.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as those commonly found in a

microbiological laboratory are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC#):	Expected Results: Plated Media:	Broth Media:
Bacillus subtilis (6633)	Growth	Good growth upon subculture
Escherichia coli (8739)	Growth, yellow zones	Good growth upon subculture, media turns yellow
Pseudomonas aeruginosa (9027)	Growth	Good growth upon subculture
Staphylococcus aureus (6538)	Growth	Good growth upon subculture
Salmonella typhimurium (14028)	Growth, yellow zones	Good growth upon subculture, media turns yellow
Aspergillus brasiliensis (16404)	Growth	Good growth upon subculture

User Quality Control: Check for signs of contamination and deterioration. Prepared plated media should appear firm, opaque, and purple in color. Broth media should appear opaque and purple in color.

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*For more detailed information, consult appropriate references.

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