

## Alternative Thioglycollate HiVeg<sup>®</sup> Medium (NIH HiVeg<sup>®</sup> Thioglycollate Broth)(Thioglycollate HiVeg<sup>®</sup> Broth, Alternative)

MV010

### Intended Use:

Recommended for sterility testing of turbid or viscous biological products and for cultivation of anaerobes and microaerophiles from various samples.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> hydrolysate	15.000
Yeast extract	5.000
Dextrose (Glucose)	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Final pH ( at 25°C)	7.1±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 29.0 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

*Note: It is preferable to use freshly prepared medium, alternatively it should be boiled and cooled just once prior to use as on reheating, toxic oxygen radicles are formed.*

### Principle And Interpretation

Alternative Thioglycollate Medium is formulated as described in the N.I.H. memorandum (1). It is used for the sterility testing of certain biological products which are turbid or viscous and cant be tested using Fluid Thioglycollate Medium (M009)(2).Both the media have similar composition, except agar and resazurin that are not included in Alternative Thioglycollate Medium. This deletion makes it suitable for sterility testing of viscous products. Alternative Thioglycollate HiVeg<sup>®</sup> Medium is same as Alternative Thioglycollate Medium except that the animal peptones are completely replaced by vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg<sup>®</sup> hydrolysate serves as a source of nitrogen and carbon compounds, long chain amino acids and other essential nutrients. Yeast extract serve as source of essential nutrients to the contaminants, if present. Dextrose serves as the energy source. Sodium chloride maintains the osmotic equilibrium of the medium whereas L-cystine, an amino acid, also serves as source of essential growth factors. Sodium thioglycollate and L-cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. Sodium thioglycollate also helps to neutralize the toxic effects of mercurial preservatives (3,4).

### Type of specimen

Pharmaceutical: Sterility testing of viscous products.

### Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility. (5)

### Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations

1. The tubes should not be reheated as frequent boiling leads to development of toxic products.
2. Prior to use the medium should be boiled once to remove the absorbed oxygen.
3. Before inoculation, the tubes should be brought to room temperature
4. The medium should not be used in fermentation process as medium contains yeast extract which is high in carbohydrate content.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Cream to yellow homogeneous free flowing powder

### Colour and Clarity of prepared medium

yellow coloured clear solution without any precipitate.

### Reaction

Reaction of 2.9% w/v aqueous solution at 25°C. pH : 7.1±0.2

### pH

6.90-7.30

### Cultural Response

Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days. (\*\*Incubated anaerobically).

Organism	Inoculum (CFU)	Growth
** <i>Clostridium sporogenes</i> ATCC 19404 (00008*)	50 -100	luxuriant
** <i>Clostridium sporogenes</i> ATCC 11437	50 -100	luxuriant
** <i>Clostridium sporogenes</i> NBRC 14293	50 -100	luxuriant
** <i>Clostridium perfringens</i> ATCC 13124 (00007*)	50 -100	luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923(00034*)	50 -100	luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50 -100	luxuriant
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50 -100	luxuriant
# <i>Pseudomonas paraaeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	luxuriant
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	luxuriant
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant
** <i>Bacteroides fragilis</i> ATCC 23745	50 -100	luxuriant
** <i>Phocaeicola</i> <i>vulgatus</i> ATCC 8482	50 -100	luxuriant

Key : \*Corresponding WDCM numbers

(#) Formerly known as *Pseudomonas aeruginosa*

\*\*\$Formerly known as *Bacteroides vulgatus*

## Storage and Shelf Life

Store between 10-30°C in tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

## Reference

1. N.I.H. Memorandum, 1955: Culture Media for Sterility Tests, 4th Revision.
2. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
3. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52: 287
4. Portwood, 1944, J. Bacteriol., 48: 255
5. The United States Pharmacopoeia, 2020. The United States Pharmacopoeial Convention. Rockville, MD.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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### Disclaimer :

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