Medium used for the cultivation and enumeration of heterotrophic organisms in treated potable water or water for injections.
*Equally use with NIER (MB-R1129N) and EP.

## - CONTENTS (Liter)

Yeast Extract 0.5 g

Proteose Peptone No. 3
0.5 g

Casamino Acid 0.5 g

Dextrose 0.5 g

Soluble Starch 0.5 g

Dipotassium Phosphate $\quad 0.3 \mathrm{~g}$
Magnesium Sulfate Heptahydrate** 0.05 g
Sodium Pyruvate $\quad 0.3 \mathrm{~g}$
Agar $\quad 15.0 \mathrm{~g}$
Final $\mathrm{pH}=7.2 \pm 0.2$ at $25^{\circ} \mathrm{C}$
**Equivalent 0.024 G Magnesium Sulfate Anhydrous

## - PROCEDURE

Suspend 18.15 G of powder in 1 L of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize by autoclave at $121^{\circ} \mathrm{C}$ for 15 minutes. Cool to $45-50^{\circ} \mathrm{C}$ in water bath. Mix well. Pour into petri dishes.

## - INTERPRETATION

R2A Agar is a medium used for the cultivation and enumeration of heterotrophic organisms in treated potable water or water for injections. Yeast extract provides vitamins. Proteose peptone no. 3 and casamino acid provide nitrogen, carbon, amino acids and minerals. Dextrose is the carbohydrate. Soluble starch neutralizes toxic metabolic by products. Dipotassium phosphate is the buffering agent. Magnesium sulfate heptahydrate is a cofactor for many metabolic reactions. Sodium pyruvate provides the recovery of stressed cells. Agar is the solidifying agent.

## - TECHNIC

Inoculate the specimen using a sterile loop to the medium. Incubate at $21 \pm 1^{\circ} \mathrm{C}$ for $72 \pm 3$ hours (*EP: 30 $35^{\circ} \mathrm{C} /$ up to 3 days). Refer appropriate references for recommended test procedure.

## - QUALITY CONTROL FOR USE

## Dehydrated medium

Appearance: free-flowing, homogeneous
Color: light beige
Prepared medium
Appearance: opalescent with a slight precipitate
Color: very light amber
Incubation conditions: $21 \pm 1^{\circ} \mathrm{C} / 72 \pm 3$ hours
(*EP: $30-35^{\circ} \mathrm{C} /$ up to 3 days)

| Microorganism | ATCC | Inoculum CFU | Growth |
| :---: | :---: | :---: | :---: |
| Enterococcus faecalis | 29212 | $50-100$ | good |
| Escherichia coli | 25922 | $50-100$ | good |
| Pseudomonas aeruginosa | 27853 | $50-100$ | good |
| Staphylococcus aureus | 25923 | $50-100$ | good |
| *Bacillus subtilis | 6633 | $50-100$ | good |
| *Pseudomonas aeruginosa | 9027 | $50-100$ | good |

## - STORE

The powder is very hygroscopic. Store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label. Store prepared medium at $2-8^{\circ} \mathrm{C}$.

## - REFERENCES

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2. APHA (1985) -Standard Methods for the Examination of Water and Wastewater 16th edition.
3. Reasoner and Geldreich. 1985. Appl. Environ. Microbiol. 49:1.
4. Fiksdal, Vik, Mills and Staley. 1982. J. Am. Water Works Assoc. 74:313.
5. Kelly, Justice and Nagy. 1983. Abstr. Q122, p. 280. Abstr. 83rd Annu. Meet. Am. Soc. Microbiol. 1983.
6. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
7. Kim and Feng. 2001. In Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. Van Soestberger and Lee. 1969. Appl. Microbiol. 18:1092.
9. Refer to the NIER.

- PACKAGE


## - MICROBIAL CULTURE IMAGES



None

S.aureus ATCC 25923

E. coli ATCC 25922

Incubation conditions: $21 \pm 1^{\circ} \mathrm{C} / 72 \pm 3$ hours

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