ALLIANCE BIO EXPERTISE

Reference: FLWR3115 **A.B.E. - Technical Data Sheet**

Product: **R2A Agar**

Specification

Solid medium for the enumeration of heterotrophic microorganisms in treated waters according to Pharmacopoeial Method.

Presentation

10 Prepared bottle
Bottle 500 ml
with: 450 ± 5 ml

Packaging Details

1 box with 10 bottles 500 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.

Shelf Life 16 months

Storage 8-25 ºC

Composition

Composition (g/l):

Proteose Peptone	0.500
Casein Peptone	0.500
Yeast extract	0.500
Glucose	0.500
Soluble starch	0.500
Sodium pyruvate	0.300
DiPotassium phosphate	0.300
Magnesium sulfate	0.024
Agar	15.000

Description /Technique

Description

R2A Agar was proposed in 1979 by Reasoner and Geldenreich and a few years later accepted by the APHA as an alternative medium for the enumeration of stressed cells in treated potable water. The culture medium has also been adopted by the European Pharmacopoeia for the control of purified water.
The use of nutrient rich media like PCA or TSA allows the growth of most microbes, but does not permit the recuperation of stressed or chlorine resistant organisms. Using a medium like R2A with low nutrients in combination with a lower temperature and longer incubation time it is possible to induce the resuscitation of these damaged cells.
In R2A Agar the source of nitrogen is the peptone and Yeast Extract supplies the vitamins and growth factors. The source of carbon is dextrose and magnesium sulfate and potassium phosphate maintain the osmotic pressure. The starch is a detoxifier and sodium pyruvate increases the recuperation of stressed cells. The agar acts as gelling agent.

Technique

The water sample must be processed as quickly as possible. If it is not possible to process within the first 6 hours, the sample must be refrigerated, but not for more than 30 hours.
R2A Agar can be used for pour plates, streak plates or filtration. The pour plate method can affect the recovery capacity of the medium because due to thermal shock when mixing molten agar with the sample. The incubating at 35ºC, an incubation period of 3-5 days is recommended. In most circumstances an incubation temperature of 20-25ºC for 5-7 days is more effective. Plates must be protected agains dehydration.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

Quality control

Physical/Chemical control

Color : Pale yellow pH: 7.2 ± 0.2 at 25ºC

Microbiological control

Melting- pour plates- Inoculate: 50-100 CFU according to the European Pharmacopoeia and according to ISO 11133 standard.

Aerobiosis. Incubation at 32,5ºC ±2,5. Reading at 24-72 h for bacteria and 5-7 days for yeasts and moulds

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Ps. aeruginosa and *E. coli* double incubation temp. 30-35 °C / 20-25 °C

Microbiological control according to ISO 11133:2014/A1:2018; A2:2020.

Microorganism	Growth
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥70%)
Staphylococcus aureus ATCC® 6538, WDCM 00032	Good (≥70%)
Bacillus subtilis ATCC® 6633, WDCM 00003	Good (≥70%)
Aspergillus brasiliensis ATCC® 16404, WDCM 00053	Good (≥70%)
Candida albicans ATCC® 10231, WDCM 00054	Good (≥70%)
Ps. aeruginosa ATCC® 9027, WDCM 00026	Good (≥70%)
E. coli ATCC® 8739, WDCM 00012 (20-25ºC)	Good (≥70%)
Ps. aeruginosa ATCC® 9027, WDCM 00026 (20-25ºC)	Good (≥70%)

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

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