Raka-Ray No. 3 Agar

SKU: 700004606, 700004607, 700004608, 700004609

NCM0192

Intended Use

Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality, and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality. It is recommended for this application by the European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC).

Contamination of beer and the beer making process by members of the lactobacilli family results in spoilage, primarily through their production of metabolic products which are detrimental to the flavor of the final product. Detection of these organisms is complicated by their diverse nutritional and environmental requirements.

A number of different formulations have been described for the isolation of lactic acid bacteria in brewing products and processes. Raka-Ray Agar was developed by the addition of various growth promoting compounds to Universal Beer Agar. This work led to the recognition that the addition of sorbitan mono-oleate, liver extract and N-acetylglucosamine produced superior growth when compared to the standard Universal Beer Agar formulation.

Further investigations provided the basis for the final formula of Raka-Ray No. 3 Medium in which fructose is an essential carbohydrate source for *Lactobacillus fructivorans.* Maltose is present to allow the growth of lactobacilli which cannot utilize glucose. The media can be made selective against yeasts by the addition of 7mg/l cycloheximide (Actidione®) and against Gram-negative bacteria by the addition of 3g/L 2-phenylethanol.

Typical Formulation

Yeast Extract	5.0 g/L
Tryptone	20.0 g/L
Liver Concentrate	1.0 g/L
Maltose	10.0 g/L
Fructose	5.0 g/L
Dextrose	5.0 g/L
Betaine HCl	2.0 g/L
Diammonium Hydrogen Citrate	2.0 g/L
Potassium Aspartate	2.5 g/L
Potassium Glutamate	2.5 g/L
Magnesium Sulphate $7H_2O$	2.0 g/L
Manganese Sulphate $4H_2O$	0.66 g/L
Potassium Phosphate	2.0 g/L
N-Acetyl Glucosamine	0.5 g/L
Agar	17.0 g/L

Final pH: 5.4 ± 0.2 at 25°C

Formula is adjusted and/or supplemented as required to meet performance specifications.

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Precaution
Refer to SDS

Preparation
1. Suspend 77.1 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.
5. If required, aseptically add 3g of 2-phenylethanol, mix well.

Test Procedure
Surface technique
Spread 0.1mL of the sample over the surface of the agar. Alternatively, the sample may be filtered and the membrane placed on the surface of the agar.

Overlay technique
Aseptically dispense 4mL volumes of Raka-Ray No.3 Agar into test tubes and keep molten at 50°C. Mix 1mL of the test sample with 4mL of molten agar and immediately pour the contents into a Petri dish containing 15-20mL Raka-Ray No.3 Agar. Mix to give isolated colonies. As the agar layer is very thin, individual colonies can be picked for further examination.
Incubate anaerobically at 25-30°C for 7 days

Quality Control Specifications
Dehydrated Appearance: Powder is a fine, free flowing, homogeneous and beige.

Prepared Appearance: Prepared medium is a clear to slightly opalescent amber colored gel.

Minimum QC:
Lactobacillus fermentum ATCC 9338
Pediococcus acidilactici NCTC 6990
Escherichia coli ATCC 25922 (inhibited / suppressed)

Results
Lactobacilli are visible after 48 hours incubation and appear as smooth, cream-colored, moist colonies approximately 1mm in diameter.
Incubation for 4 days may be sufficient, however slow-growing organisms such as *Pediococcus* may require up to 7 days.
If the number of colonies on the plate exceeds 300, dilute the sample 1:10 in Maximum Recovery Diluent (NCM0085) and retest.
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Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

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Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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