

Technical Information

K.R.A.N.E.P. Agar Base

Product Code: DM 1583

Application: - K.R.A.N.E.P. Agar Base is used for selective enumeration of total Staphylococci from food stuffs.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Yeast extract	1.500
Beef extract	1.500
Potassium thiocyanate	25.500
Sodium pyruvate	8.200
Mannitol	5.100
Lithium chloride	5.100
Sodium azide	0.050
Cycloheximide	0.041
Agar	15.000
Final pH (25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The name K.R.A.N.E.P. Agar comes from the initial letters of its main diagnostic, selective and stimulatory agents like Kalium-Rhodanid-Actidione-Natriumazid-Eigelb-Pyruvate is a selective medium used for the enumeration of Staphylococcus aureus in foods, which was first described, by Sinell and Baumgart ⁽¹⁾.

Potassium thiocyanate and mannitol make the medium selective for the detection of Staphylococci ⁽²⁾ which is further enhanced by the addition of sodium azide and cycloheximide ⁽³⁾. Sodium pyruvate and egg yolk emulsion added to the medium serve as growth enhancer and diagnostic agent respectively ^(4, 5). K.R.A.N.E.P. Agar is recommended for the selective isolation of coagulase negative Staphylococci from meat products ^(6, 7) and therefore this medium is used to enumerate the total staphylococcal count both coagulase positive and coagulase negative Staphylococci, from food products.

Peptic digest of animal tissue, yeast extract and beef extract in the medium supplies essential growth nutrients including B complex nutrients. Cycloheximide inhibits most of the yeasts and moulds. Inclusion of sodium azide helps to inhibit the growth of accompanying aerobic organisms like Bacillus species, which interfere with the cultivation of Staphylococci ⁽⁸⁾. The presences of inhibitory agents fail various gram-negative bacteria as well as gram-positive bacteria to grow on this medium ^(4, 9).

Inoculation can be done by spread plate technique using 0.1 ml inocula on Petri plates or 0.05 ml each from different tentold dilution steps in drop plate technique. After incubation of 48 hours, well-grown golden yellow colonies with a precipitation zone of egg yolk in the medium, which remains opaque, are considered as S.aureus. Confirmatory tests for coagulase production are required. Colonies typical for S.aureus but without an egg yolk reaction should also be tested for coagulase and if positive their identity is confirmed by further tests ⁽¹⁰⁻¹²⁾.

Methodology

Suspend 71.99 grams of powder media in 900 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add sterile 100 ml of Egg Yolk Emulsion (MS2045). Mix well and pour into sterile Petri plates.

Warning: Cycloheximide is very toxic and lithium chloride is harmful. Avoid skin contact or aerosol formation and inhalation. Sodium azide has a tendency to form explosive metal azides with plumbing materials, use enough water to flush of the disposables.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Light yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion : Yellow coloured opaque gel forms in Petri plates.

pH Range 6.60-7.00

Cultural Response/ characteristics

DM 1583: Cultural characteristics observed with added sterile Egg Yolk Emulsion (MS2045) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics	Lecithinase activity
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant	>=50%	golden shiny	positive, opaque zone around the colony
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	luxuriant	>=50%	white shiny	negative
<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited	0%		
<i>Candida albicans</i> ATCC 10231	>=10 ³	inhibited	0%		
<i>Bacillus subtilis</i> ATCC 6633	>=10 ³	inhibited	0%		

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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12. Devriese L. A., and Hajek V., 1980, J. Appl. Bacteriol., 49, 1- 11.

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