PRODUCT SPECIFICATION SHEET

K.R.A.N.E.P. Agar Base (DM125)

Intended Use
K.R.A.N.E.P. Agar Base (DM125) is recommended for selective enumeration of total *Staphylococci* from foodstuffs.

Product Summary and Explanation
K.R.A.N.E.P. Agar is a selective medium first described by Sinell and Baumgart[1] for the enumeration of *Staphylococcus aureus* in foods. The name K.R.A.N.E.P. Agar comes from the initial letters of its important diagnostic, selective and stimulatory agents like Kalium-Rhodanid-Actidione-Natriumazid-Eigelb-Pyruvate. The presence of potassium thiocyanate and mannitol make the medium selective for *Staphylococci*. [2] The selectivity is further enhanced by the addition of sodium azide and cycloheximide.[3] Sodium pyruvate serves as a growth enhancer and egg yolk emulsion added to the medium acts as a diagnostic agent.[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 i.e. coagulase positive and coagulase negative *Staphylococci*, from food products.

Principles of the Procedure
K.R.A.N.E.P. Agar Base contains peptic digest of animal tissue, yeast extract and beef extract which provides carbon, nitrogen and amino acid sources including B complex nutrients essential for growth. Cycloheximide inhibits most of the yeasts and moulds. Sodium azide helps to inhibit the accompanying aerobic organisms like *Bacillus* species, which interfere with the cultivation of *Staphylococci*. Due to the presence of inhibitory agents, various gram-negative bacteria as well as gram-positive bacteria fail to grow on this medium.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Yeast extract	1.50
Beef extract	1.50
Potassium thiocyanate	25.50
Sodium pyruvate	8.20
Mannitol	5.10
Lithium chloride	5.10
Sodium azide	0.05
Cycloheximide	0.041
Agar	15.00
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Cycloheximide is very toxic and lithium chloride is harmful. Avoid skin contact or aerosol formation and inhalation.
4. Sodium azide has a tendency to form explosive metal azides with plumbing materials, use enough water to flush off the disposables.

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Directions

1. Suspend 71.99 grams in 900 ml distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C and aseptically add sterile 100 ml of Egg Yolk Emulsion (MS038).
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Light yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion : Yellowcoloured opaque gel forms in Petri plates.
Reaction of 7.2% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added sterile Egg Yolk Emulsion (MS038) after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Colony Characteristics	Lecithinase Activity
1.	*Staphylococcus aureus ATCC 25923*	50 -100	good-luxuriant	>=50%	golden shiny	positive, opaque zone around the colony
2.	*Staphylococcus epidermidis ATCC 12228*	50 -100	good-luxuriant	>=50%	white shiny	negative
3.	*Escherichia coli ATCC 25922*	$>=10^3$	inhibited	0%	--	--
4.	*Candida albicans ATCC 10231*	$>=10^3$	inhibited	0%	--	--
5.	*Bacillus subtilis ATCC 6633*	$>=10^3$	inhibited	0%	--	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculation can be done by spread plate technique using 0.1 ml inocula on Petri plates or 0.05 ml each from different decimal dilution steps in drop plate technique.
2. Refer to appropriate references for test procedures for selective enumeration of total *Staphylococci* from foodstuffs.

Results

1. *S.aureus* is considered positive if well-grown golden yellow colonies with a precipitation zone of egg yolk is observed in the medium, which remains opaque.
2. 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 should be confirmed by further tests.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : K.R.A.N.E.P. Agar Base
Product Code : DM125
Available Pack sizes : 100 gm

References

1. Sinell H. J. and Baumgart J., 1967, Zbl. Bakt. I. Abt. Orig., 204:248.
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3. Sinell H. J. and Baumgart J., 1965, Zent. Bl. Bakt. I. Abt. Orig., 197:447.
4. Baird - Parker A. C., 1962, J. Appl. Bacteriol., 25:12.
5. Gillespie W. A. and Alder V. G., 1952, J. Pathol. Bacteriol., 64:187.
6. Sinell H. J. and Kusch D., 1969, Arch. Hyg. (Berlin), 153:56-66.
7. Sinell H. J., Kusch D., and Untermann F., 1970, Zent. Bl. Veterinarmed Reihe., 17: 429-435.

Further Information

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