

TM 1206 - K.R.A.N.E.P. AGAR BASE

INTENDED USE

For selective enumeration of total Staphylococci from foods.

PRODUCT SUMMARY AND EXPLANATION

K.R.A.N.E.P. Agar is a selective medium used for the enumeration of *Staphylococcus aureus* in foods, which was first described, by Sinell and Baumgart. 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. The medium is selective for the detection of Staphylococci due to the presence of potassium thiocyanate and mannitol. The selectivity is further enhanced by the addition of sodium azide and cycloheximide. Sodium pyruvate and egg yolk emulsion added to the medium serve as growth enhancer and diagnostic agent respectively. K.R.A.N.E.P. Agar is recommended for the selective isolation of coagulase negative Staphylococci from meat products and therefore this medium is used to enumerate the total staphylococcal count i.e. coagulase positive and coagulase negative Staphylococci, from food products.

Inoculation can be done by spread plate technique using 0.1 ml inoculate on Petri plates or 0.05 ml each from different decimal 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 should be confirmed by further tests.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	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

PRINCIPLE

Peptone, 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 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.

INSTRUCTION FOR USE

- Dissolve 71.99 grams in 900 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.



- Cool to 45-50°C and aseptically add sterile 100 ml of Egg Yolk Emulsion.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance 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 (at 25°C) : 6.8±0.2

INTERPRETATION

Cultural characteristics observed with added sterile Egg Yolk Emulsion after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colony characteristics	Lecithinase	Incubation Temperature	Incubation Period
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	25923	50-100	Luxuriant	≥70%	Golden shiny	Positive, opaque zone around the colony	35 - 37°C	24-48 Hours
<i>Staphylococcus epidermidis</i>	12228	50-100	Luxuriant	≥70%	White shiny	Negative	35 - 37°C	24-48 Hours
<i>Escherichia coli</i>	25922	≥10 ³	Inhibited	0%	-	-	35 - 37°C	24-48 Hours
<i>Candida albicans</i>	10231	≥10 ³	Inhibited	0%	-	-	35 - 37°C	24-48 Hours
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	6633	≥10 ³	Inhibited	0%	-	-	35 - 37°C	24-48 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.










DISPOSAL



After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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 GMP Good Manufacturing Practices Certified	 Best Before	 QTY. Quantity	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 LOT/ B. NO. Lot / Batch Number	 Consults Instructions for Use	 QR Code	

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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