

TM 1364 - KOHN TWO TUBE MEDIUM NO. 2

INTENDED USE

For identification of *Enterobacteriaceae* on the basis of sucrose and salicin fermentation, motility, hydrogen sulphide and indole production.

PRODUCT SUMMARY AND EXPLANATION

Russell first introduced Double Sugar Medium, a differentiating medium for *Enterobacteriaceae*. Kohn later developed a technique employing two tubes of composite media for study of culture reactions, for the identification of *Enterobacteriaceae*. Gillies further made minor modifications in Kohns media. Kohn Two Tube Medium No.2 is used to study carbohydrate fermentation (Sucrose and Salicin) along with motility, hydrogen sulfide production and indole production.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Tryptone	10.000
Saccharose (Sucrose)	10.000
Salicin	10.000
Sodium chloride	5.000
Sodium thiosulphate	0.016
Disodium hydrogen phosphate	0.090
Bromothymol blue	0.020
Agar	3.000

PRINCIPLE

Peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins provides essential nutrients. Sodium chloride maintains the osmotic balance of the medium. Tryptone is an enzymatic digest of casein used as a nitrogen source in culture media.

INSTRUCTION FOR USE

- Dissolve 48.13 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Dispense in tubes. Sterilize by autoclaving at 115°C (10 psi pressure) for 15 minutes.
- Cool the tubed medium in an upright position.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder** : Cream to yellow homogeneous free flowing powder.
- Appearance of prepared medium** : Green coloured, clear to slightly opalescent gel forms in tubes as butts.
- pH (at 25°C)** : 7.4±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.



Microorganism	ATCC	Inoculum (CFU/ml)	Motility	Fermentation w/ Sucrose/ Salicin	H ₂ S (with lead Indole acetate strip)	Indole	Incubation Temperature	Incubation Period
<i>Proteus vulgaris</i>	13315	50-100	Positive, growth away from stabline causing turbidity	Acid & gas production or negative reaction	Variable reaction	Variable reaction	35-37°C	18-24 Hours
<i>Salmonella Typhimurium</i>	14028	50-100	Positive, growth away from stabline causing turbidity	Negative reaction	Variable reaction	Negative reaction	35-37°C	18-24 Hours
<i>Salmonella Typhi</i>	6539	50-100	Positive, growth away from stabline causing turbidity	Negative reaction	Positive, blackening of the lower portion of the strip	Negative reaction	35-37°C	18-24 Hours
<i>Shigella flexneri</i>	12022	50-100	Negative, growth along the stabline, surrounding medium remains clear	Negative reaction	Negative, no blackening	Variable	35-37°C	18-24 Hours
<i>Shigella sonnei</i>	25931	50-100	Negative, growth along the stabline, surrounding medium remains clear	Negative reaction	Negative, no blackening	Negative reaction	35-37°C	18-24 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

- Gillies R. R., 1956, J. Clin. Pathol., 9(4):368.
- Isenberg, H. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
- Kohn J., 1954, J. Path. Bacteriol., 67(1): 286.
- Russell F. F., 1911, J. Med. Res., 25:217.



 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Buckenhof 10 48163 Aachen, Germany</small>	 European Conformity	 QR Code	 Consults instructions for Use	 Best Before

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

***For Lab Use Only**
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