TM 752 – LACTIC ACID BACTERIA SELECTIVE AGAR BASE

INTENDED USE
For selective isolation of lactic acid bacteria from brewery.

PRODUCT SUMMARY AND EXPLANATION
Lactic Acid Bacteria Selective Agar Base is based on the formula of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes. It is recommended by European Brewing convention (EBC) and the American Society of Brewing Chemists for isolation of Lactobacilli. The family *Lactobacillaceae* has members that are important spoilage organisms in the brewing process. The original medium viz. Raka-Ray Medium was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci. Further studies towards optimization of conditions of growth factors led to the modification of Raka Ray medium with the addition of sorbitan mono-oleate to stimulate growth of lactic acid bacteria and incorporation of sugars such as fructose as an essential carbohydrate source for Lactobacillus fructivorans and maltose for lactobacilli as it lacks the ability to metabolize glucose.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	5.000
Tryptone	20.000
Liver concentrate	1.000
Maltose	10.000
Fructose	5.000
Dextrose (Glucose)	5.000
Betaine hydrochloride	2.000
Diammonium hydrogen citrate	2.000
Potassium aspartate	2.500
Potassium glutamate	2.500
Magnesium sulphate	2.000
Manganese sulphate	0.660
Potassium dihydrogen phosphate	2.000
N-acetyl glucosamine	0.500
Agar	17.000

PRINCIPLE
This medium consists of Tryptone which provides the nitrogenous compounds, potassium aspartate and potassium glutamate are additional sources of the respective amino acids while diammonium hydrogen citrate buffers the medium. The addition of phenylethanol and cycloheximide in the supplement make the medium selective for the isolation of lactic acid bacteria in beer. Phenylethanol inhibits gram-negative organisms, while yeasts are inhibited by cycloheximide. Polysorbate 80 or sorbitan monooleate, Liver concentrate, yeast extract and N-acetyl glucosamine act as growth stimulating agents. Fructose is the essential carbohydrate source for *Lactobacillus fructivorans*, maltose helps in detection of lactobacilli which cannot utilize glucose whereas glucose is utilized by pediococci.

INSTRUCTION FOR USE

- Dissolve 38.58 grams in 500 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 contents of 1 vial of Lactic supplement.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to beige homogeneous free flowing powder.
Appearance of prepared medium : Dark amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 5.4 ± 0.2

INTERPRETATION

Cultural characteristics observed under anaerobic condition, with added Lactic Supplement after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Lactobacillus acidophilus	11506	50-100	Good-luxuriant	>=50%	25-30°C	18-48 Hours
Lactobacillus plantarum	8014	50-100	Good-luxuriant	>=50%	25-30°C	18-48 Hours
Lactobacillus fermentans	9338	50-100	Good-luxuriant	>=50%	25-30°C	18-48 Hours
Lactobacillus brevis	367	50-100	Good-luxuriant	>=50%	25-30°C	18-48 Hours
Lactobacillus buchneri	11307	50-100	Good-luxuriant	>=50%	25-30°C	18-48 Hours
Pedicoccus acidilactis	8042	50-100	None-poor	0-10%	25-30°C	18-48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	25-30°C	18-48 Hours
Saccharomyces cerevisiae	9763	>=10^3	Inhibited	0%	25-30°C	18-48 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.

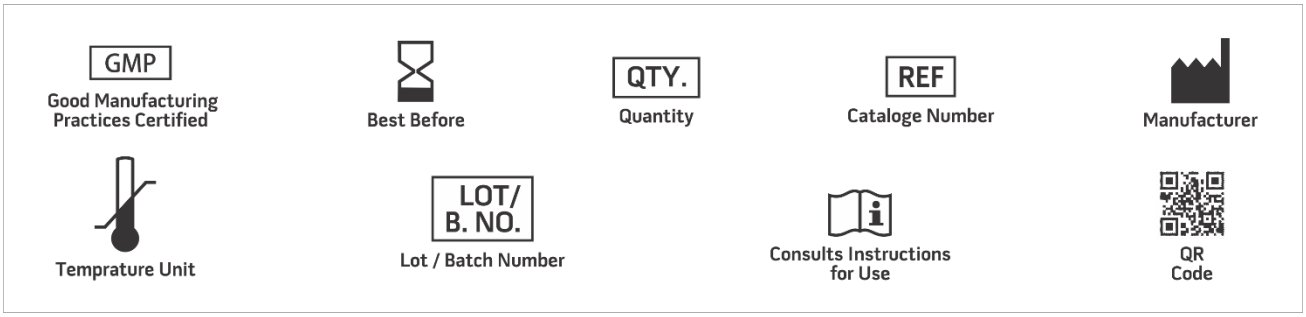
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

REFERENCES

1. Coster E. and White H.R. (1951) J. Gen. Microbiol. 37.15.
2. European Brewing Convention, EBC Analytical Microbiologica: Part II J. Inst. Brewing (1981) 87. 303-321.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Lawrence D.R. and Leedham P.A. (1979) J. Inst. Brewing 85. 119.
6. Methods of Analysis of the ASBC (1976) 7th Edition. The Society, St. Paul Mn USA.
7. Saha R.B., Sondag R.J. and Middlekauff J.E. (1974) Proceedings of the American Society of Brewing Chemists, 9th Congress, 1974.
8. Van Keer B., Van Melkebeke L., Vertriest W., Hoozee G. and Van Schoonenberghe E. (1983) J. Inst. Brewing 89. 361-363.



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

***For Lab Use Only**

Revision: 08 Nov., 2019