User Manual

LMDA

Item No. 9.23555.244

1. Information

LMDA - Lee´s Multi-Differential Agar (pH 5.5-6.5) is a complex nutrient medium that will detect most organisms commonly encountered in a brewery. It is a ready-to-use powder for the preparation of LMDA Agar which is used for the detection and enumeration of bacteria and yeast.

Principle:
This medium contains solid calcium carbonate ($CaCO_3$) which gets dissolved by the acid released by acid-producing bacteria during growth. Furthermore, identification of the colonies will be facilitated by characteristic color reactions.
Note: Cycloheximide can be added to suppress growth of culture yeas, to make this media selective to bacteria.

2. Handling

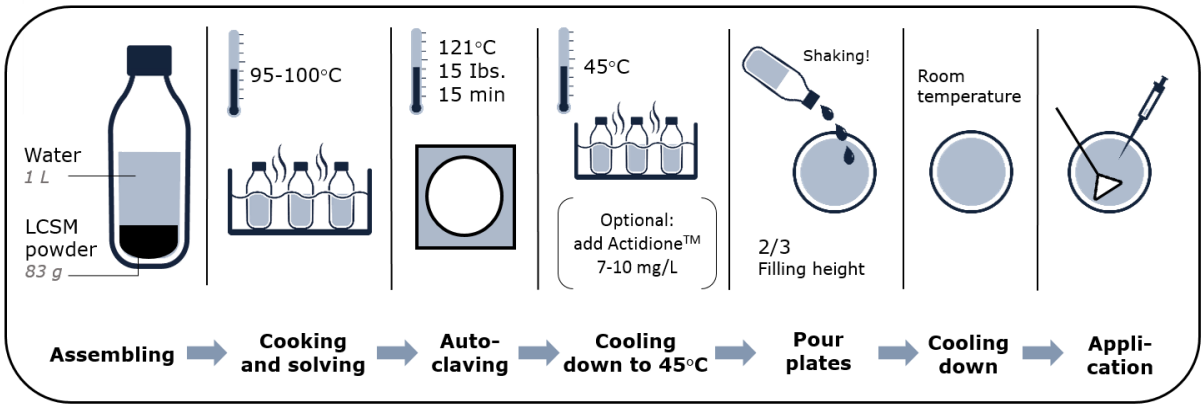
2.1 Preparation and Application

Please work under sterile conditions to avoid secondary contamination of the samples after autoclaving.

Suspend 83 grams of LMDA in 1 L distilled water and seal with an appropriate permeable closure. Dissolve the medium completely by bringing to boil and continue boiling for one minute with constant agitation. If direct heat is used, avoid scorching. Autoclave medium at 121°C for 15 minutes for sterilization. A higher temperature and/or longer autoclaving time are detrimental to the medium. Transfer the medium to a water bath at 45-50°C as soon as possible after sterilization.

If selectivity for bacteria is needed the addition of Cycloheximid is necessary. Cycloheximid should be added after autoclaving at the rate of 7-10 mg per liter of LMDA solution. It is suggested that 70-100 mg of Cycloheximid be dissolved in 100 ml of sterile water and that 10 ml of the Cycloheximid solution be used subsequently per liter of LMDA.

After cooling to 45-50°C, re-suspend the insoluble, sedimented $CaCO_3$ by swirling or gently shaking the flask while avoiding creation of excessive foam. Medium can be poured afterwards in sterile petri dishes. Fill until 2/3 of dish height is reached and allow the medium to solidify. While pouring, swirl the flask frequently to keep $CaCO_3$ in suspension. To ensure an even distribution of $CaCO_3$, avoid moving the dishes once poured. If any foam remains at the top of the medium after pouring, quickly flame plates with a Bunsen burner to break bubbles and ensure a smooth and even surface. Plates can be stored maximum 2 weeks in inverted position at 4°C until required for use.



2.2 Application

Application is performed as follows:

1. Dilute the sample, as necessary, to obtain dilutions containing between 100 and 900 bacteria cells/ml.
2. Pipet 0.1 to 0.2 ml aliquots of the sample or its dilution onto the cooled agar for inoculation and disperse sample evenly with a sterile spreader.

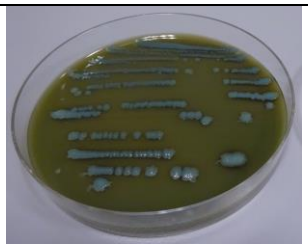
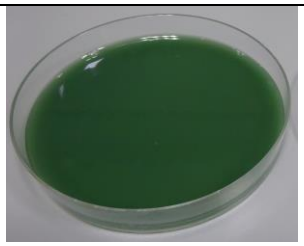
2.3 Incubation

Incubate the samples in inverted position, in an incubator under anaerobic conditions (for beer spoiling bacteria) or aerobic conditions (for yeast and wort spoiling bacteria) for 4 to 7 days at a temperature of 30°C. It may be possible to observe the growth of some bacterial colonies earlier, but the morphological characteristics may not be completely developed.

3. Evaluation and Interpretation of the Results

Count colonies on all plates and identify strains by morphological appearance. If in doubt, Gram stain, catalase and/or oxidase test can be used.

Special Features of Evaluation

Occurring Result		Valuation
Green opaque agar with green-bluish colonies		Positive finding *Acetobacter pasteurianus*
Green, opaque Agar		Negative finding

4. Product Information

4.1 Packaging and Content

Item Item no.	Packaging Content	Size [cm]	Weight [kg] netto	 gross
LMDA Pulver 9.23555.244	1x 500g powder in PE bottle	21,5 x 8 x 8	0,50	0,59

4.2 Storage and Shelf Life

Store at 4-8°C under dry and dark conditions according to product specification.

4.3 Waste Disposal

- No dangerous good
- No hazardous material
- Please consider your local waste regulations
- Not inoculated media can be disposed of with normal laboratory waste
- Inoculated and incubated media are to be sterilized before disposal at a temperature of 121°C/250°F for 20 min

4.4 Warnings and Precautions

Always wear protective clothing when handling hot media (see safety data sheet).
Be careful when working with hazardous material. Please consider safety data sheet.
This product is only for use in microbiological control. Do not use for medical purpose or use.

5. Quality Control

LMDA (Lee´s Multi-Differential Agar) was tested with following microorganisms for the microbiological quality control.

5.1 Tested Microorganisms (at 30°C after 3-5 days)

Microorganism	Growth aerobic	Growth anaerobic
Lactobacillus brevis (BRY 406)	Light blue/green, shiny 1-2mm colonies, halo of clearing (acidification)	Light blue/green with dark green center to dark green, shiny, 1-2mm colonies, acidification
Pediococcus damnosus (BRY 407)	+ or -	Opaque light blue shiny colonies with a halo of clearing (1-2 mm)
Klebsiella aerogenes (DSM30053)	+	Opaque, light blue matt colonies with acidification (2 mm)
Acetobacter pasteurianus (BRY488)	Grey/ blue-green shiny colonies (1-2 mm)	No growth
Saccharomyces cerevisiae (BRY420)	Cream to pale green matt colonies (2-4 mm)	Not specified

6. Similar Products

Product	Item No.	Targeted Organism	Sample Type
NBB A	2.04709.782	Beer spoiling bacteria	Beer
HLP	9.23556.244	Beer spoiling bacteria	Beer

7. References

[1] ASBC (2008): Differential Culture Media (Microbiological Control-5). Edition 14.