

MRS AGAR WITH TWEEN® 80
MRS BROTH WITH TWEEN® 80

Powdered and ready to use media for the cultivation of lactic acid bacteria

TYPICAL FORMULAS**MRS AGAR (g/L)**

Peptone	10
Beef Extract	10
Yeast Extract	5
Glucose	20
Di-potassium Hydrogen Phosphate	2
Sodium Acetate	5
Di-amonium Citrate	2
Magnesium Sulphate	0.2
Manganous Sulphate	0.05
Agar	15
Tween® 80	1

MRS BROTH (g/L)

Peptone	10
Beef Extract	10
Yeast Extract	5
Glucose	20
Di-potassium Hydrogen Phosphate	2
Sodium Acetate	5
Di-amonium Citrate	2
Magnesium Sulphate	0.2
Manganous Sulphate	0.05
Tween® 80	1

DIRECTIONS FOR POWDERED MEDIA

Suspend 70.2 g of MRS Agar or 55.2g of MRS Broth in 1000 mL of cold distilled water, heat to boiling. Distribute into bottles/tubes of appropriate capacity and sterilise in the autoclave at 121 °C for 15 minutes.

Final pH 6.4 ± 0.2

DESCRIPTION

MRS Agar with Tween® 80 and MRS Broth with Tween® 80 prepared according to the formula of De Man, Rogosa and Sharpe, are designed for the cultivation, enrichment and isolation of *Lactobacillus* spp. from all types of materials. Prepared without the tomato extract, the media seem to be particularly sensitive in the isolation of lactobacilli from milk and dairy products. However, lactobacilli of any origin grow on these media. The Tween® 80, sodium acetate and diammonium citrate intensifies the growth of lactobacilli. As these media exhibit a poor degree of selectivity, *Pediococcus* and *Leuconostoc* spp. and other secondary bacteria may grow on them.

TECHNIQUE

For the enumeration of mesophilic lactic acid: prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned.

1. Transfer by means of sterile pipettes 1mL of the test sample (if liquid) or 1mL of the initial suspension and 1mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
2. Pour approximately 15 mL of MRS Agar with Tween 80, cooled to approximately 47 °C into each dish
3. Mix well with the inoculum the medium and allow the mixture to solidify
4. Incubate at 30 °C for 72-hrs ± 3 h. Longer incubation may result in excess blackening along the bottom rim of the plates.
5. Count the colonies on the plates containing between 15 and 300 colonies.

If lactic acid bacteria other than mesophilic are to be enumerated, incubate the plates at 42 °C for 48hrs (thermophilic lactobacilli) or at 25 °C for 5 days (psicrofilic lactobacilli) or at 30 °C for 48hrs + 22 °C for 48 hrs (mesophilic+psicrofilic lactobacilli). Some *Leuconostoc* spp. may form large slimy colonies, which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria. *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, can grow on MRS Agar. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine. Due to the possible development of microorganisms other than lactic acid bacteria on MRS Agar, it may be necessary in some cases and for some products to confirm the colonies by a simple technique - Gram staining and catalase.

MRS Broth may be used as it is for the cultivation of lactic acid bacteria or supplemented with agar, as a solid culture medium for the detection of lactobacilli.

USER QUALITY ASSURANCE (37°C-48HRS)

Productivity control

L.fermentum ATCC 9338*L.sake* ATCC 15521**STORAGE**

Dehydrated medium: 2-8 °C °C

User prepared plates: 15 days at 2-8 °C

User prepared flasks or tubes: 15 days at 2-8 °C

Ready to use plates: 2-8 °C °C

REFERENCES

- Briggs M. (1953) J. Dairy Res., **20**, 36.
- Cox, G.P. & Briggs M. (1954) J. App. Bact. **17**, 18.
- De Man, J.C., Rogosa, M. & Share, M.E. (1960).. J. Appl. Bact . **23**, 130-135.

PACKAGING

4017282	MRS Agar with Tween® 80	500g (7,1 L)
4017284	MRS Agar with Tween® 80	5kg (71 L)
4017292	MRS Broth with Tween® 80	500g (9,1 L)
4017294	MRS Broth with Tween® 80	5kg (91 L)
541728	MRS Agar with Tween® 80	20 ready to use plates