# Instructions for Use

## REF - KM0030 de Man, Rogosa and Sharpe (MRS) Broth

Dehydrated Culture Media

### 1. Intended Use

KM0030 de Man, Rogosa and Sharpe (MRS) Broth is used for the cultivation and enumeration of *Lactobacillus* species from a variety of sources by the method described by Miles and Misra [1]. It may be used in conjunction with MRS Agar (E&O KM0080 or PP0954).

### 2. Composition*

| Ingredient | g/L |
|---|---|
| Peptones | 9.75 |
| Yeast extract | 4.0 |
| Beef extract | 8.0 |
| Glucose | 20.0 |
| Dipotassium hydrogen phosphate | 2.0 |
| Sodium acetate | 5.0 |
| Ammonium citrate | 2.0 |
| Magnesium sulphate | 0.2 |
| Manganese sulphate | 0.05 |
| Polysorbate 80 | 1.0 |

*Adjusted/supplemented as needed to meet performance requirements

### 3. Summary and Explanation

De Man, Rogosa and Sharpe (MRS) Broth is based on the formulations of de Man, Rogosa and Sharpe [2]. This medium was shown to support good growth of all lactobacilli from the oral cavity, faecal matter, dairy products, and other sources. MRS broth can be used to determine whether Gram-positive bacilli produce gas during glucose fermentation. This is helpful to differentiate gas producing *Leuconostoc* spp. from *Lactobacillus* spp., which are not. A Durham tube may be added to the MRS broth to detect gas production; displacement of broth in the Durham tube suggests gas production. MRS medium is particularly recommended for the enumeration and maintenance of lactobacilli by the most probable number (MPN) technique [3].

### 4. Principle

Peptones, beef extract, and yeast extract provide the required nitrogen and other essential nutrients. Glucose is fermented to provide energy and dipotassium hydrogen phosphate is a buffering agent to help maintain pH. Selectivity of the medium is achieved using ammonium citrate and sodium acetate, inhibiting microorganisms such as *streptococci* and moulds. The magnesium sulphate, manganese sulphate and polysorbate 80 function as growth stimulants. The medium can be made more selective for lactobacilli by lowering the pH to between 5.0 and 5.5. This has the effect of inhibiting most streptococci that may otherwise grow in the broth and can be readily confused with lactobacilli [4].

Bacterial strains that can ferment glucose will overcome the selective agents and produce good growth. Certain organisms such as *Leuconostoc* spp., produce gas in MRS medium. This can be a useful tool to differentiate these organisms from non-producers of gas such as *Lactobacillus* spp.

### 5. Preparation Instructions

Suspend 52.0g of dehydrated culture medium in 1 litre of deionised/purified water and allow to mix for 10 minutes.
Dispense into appropriate containers then autoclave to sterilise at 121°C for 15 minutes.

### 6. Physical Characteristics

| | Dehydrated Medium | Prepared Medium |
|---|---|---|
| Appearance and Colour | Straw fine powder | Dark straw liquid |
| pH | N/A | 6.5 ± 0.2 |

### 7. Materials Provided

KM0030 is available in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

| Product Code | Product Format |
|---|---|
| KM0030-500G-500 | 1 x 500g Dehydrated Culture Media Tub |
| KM0030-5KG-5000 | 1 x 5kg Dehydrated Culture Media Tub |
| KM0030-10KG-10000 | 1 x 10kg Dehydrated Culture Media Tub |

## 8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

## 9. Specimens

KM0030 de Man, Rogosa and Sharpe (MRS) Broth is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption and environmental samples.
- Already isolated colonies from agar.
- Samples from Humans and Animals for research purposes only: faecal matter, samples from oral cavity or any other area of interest.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

## 10. Test Procedures and Interpretation of results

Follow local guidelines.

Food and Environmental:

If necessary, homogenize the sample material using a ratio of 1:10 of sample to de Man, Rogosa and Sharpe (MRS) Broth (serial dilutions may also be made) for enrichment or for determining the bacterial count by the MPN method [3].

Specimens from Humans and Animals:

For research purposes, faecal and other human/animal samples may be added directly to the broth. Faeces may also be diluted 1:4 in sterile saline solution or 0.1% peptone water before adding 1ml to the broth.

Isolated colonies:

Inoculate MRS broth lightly with an 18- to 24-hour culture from agar or broth.

For the testing of gas production, use a broth bottle to which a Durham tube has been added.

Incubate at 37 ± 1°C in 5% $CO_2$ for 48-72 hours.

After incubation, examine broths for turbidity and gas production where applicable; examine subcultures on agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of isolated from subcultures. Refer to relevant local guidelines.

## 11. Quality Control

| Organism | Incubation | Result (Specificity) |
|---|---|---|
| *L. sakei* (NCTC 13842) | 37 ± 1°C in 5% $CO_2$ for 48-72 hours | Broth: Turbid. Subculture: White colonies |
| *L. lactis* (NCTC 6681) | 37 ± 1°C in 5% $CO_2$ for 48-72 hours | Broth: Turbid. Subculture: White colonies |
| *L. brevis* (NCTC 13386) | 37 ± 1°C in 5% $CO_2$ for 48-72 hours<br>Subculture 1µl onto E&O PP0120 Columbia Blood Agar and incubate at 37 ± 1°C in 5% $CO_2$ for 48-72 hours. | Broth: Turbid. Subculture: White colonies |

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

## 12. Performance

To fully verify KM0030 de Man, Rogosa and Sharpe (MRS) Broth performance, dehydrated culture medium samples were used to prepare MRS Broth (10ml) and tested for turbidity, as well as colony morphology when 1µl on incubated culture in MRS Broth was subcultured onto E&O PP0120 Columbia Blood Agar. Prepared broth samples were incubated at 37 ± 1°C in 5% $CO_2$ for 48-72 hours, and PP0120 subcultures were incubated at 37 ± 1°C in 5% $CO_2$ for 48-72 hours. All samples of prepared medium showed expected recovery and morphology of the required test organisms: *Latilactobacillus sakei* (NCTC 13842), *Lactococcus lactis* (NCTC 6681) and *Lactobacillus brevis* (NCTC 13386). Therefore, it can be concluded that KM0030 de Man, Rogosa and Sharpe (MRS) Broth, meets performance criteria when used according to the instructions outlined above.

## 13. Limitations of the Media

- Organisms other than lactobacilli may grow in this media.
- Due to natural variation, some strains may grow poorly in this medium.

## 14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0030 Material Safety Data Sheet.

## 15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

## 16. References


1. Miles, A., Misra, S. and Irwin, J. (1938) The estimation of the bactericidal power of the blood. The Journal of hygiene, 38(6), pp.732–749.
2. de Man, J, Rogosa, M and Sharpe, M. (1960) A medium for the cultivation of lactobacilli. Journal for Applied Bacteriology, 23, pp.130-135.
3. Austin, J. and Pagotto, F. (2003) MICROBIOLOGY | Detection of Foodborne Pathogens and their Toxins in Encyclopedia of Food Sciences and Nutrition, 2nd ed., pp.3886-3892. Academic Press.
4. Reuter, G. (1985) Elective and selective media for lactic acid bacteria. International Journal of Food Microbiology, 2(1-2), pp.55-68.


## Version History*

001 18/09/23 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.


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**TABLE OF APPLICABLE SYMBOLS**

| | | | | |
|---|---|---|---|---|
| REF o REF Catalogue number | LOT Batch code | | Manufacturer | Use by |
| Temperature limitation | Contents sufficient for <n> tests | Consult Instructions for Use | Keep away from direct light | Store in a dry place |