

**MR - VP BROTH**

Medium for Gram-negative bacteria differentiation, recommended by ISO 6785: 2001.

**TYPICAL FORMULA (g/L)**

Tryptone	3.5
Peptone	3.5
Glucose	5.0
Potassium Phosphate Monobasic	1.7
Potassium Phosphate Bibasic	3.3
Final pH	7.0 ± 0.2

**DESCRIPTION**

MR-VP BROTH is a medium for Gram-negative bacteria differentiation based on the Methyl Red and Voges- Proskauer tests, recommended by ISO 6785: 2001.

**PRINCIPLE**

Tryptone and peptone are sources of carbon, nitrogen, aminoacids and proteins. Glucose is the substrate for fermentation. Potassium phosphate is the buffer of the medium. Methyl red-positive organisms produce high levels of acid during fermentation of glucose, overcome the phosphate buffer system and produce a red color upon the addition of the methyl red pH indicator. In the Voges-Proskauer test, the red color produced by the addition of potassium hydroxide to cultures of certain microbial species is due to the ability of the organisms to produce a neutral end product, acetoin (acetyl methylcarbinol), from the fermentation of glucose. The acetoin is oxidized to diacetyl in the presence of oxygen and alkali (KOH). Diacetyl, under the catalytic action of  $\alpha$ -naphthol and creatine, is converted into a red complex.

**TECHNIQUE**

Using a light inoculum, inoculate tubes of MR-VP media with 18- to 24-hour pure cultures. Incubate tubes aerobically at  $35 \pm 2^\circ\text{C}$  for a minimum of 48 hours but preferably for 5 days.

Prepare the methyl red indicator by dissolving 0.1 g of methyl red in 300 mL of 95% ethyl alcohol. Add sufficient purified water to make 500 mL.

After the appropriate incubation period, aseptically remove aliquots (1 mL for the VP test) of the medium and conduct the following tests:

1. Methyl Red Test – Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the color result immediately.
2. Prepare the following solution:
  - creatine monohydrate: 0.5 g in 100 mL of distilled water ;
  - 5%  $\alpha$ -naphthol in ethyl alcohol 96%;
  - 40% KOH in distilled water.

To 3 mL of broth culture add 2 drops of creatine solution, 3 drops of  $\alpha$ -naphthol solution and 2 drops of KOH solution. Gently shake the tube to expose the medium to oxygen. A positive test is indicated by the development of a pink to bright red coloration 15 minutes after the addition of reagents.

**INTERPRETATION OF RESULTS**

Methyl Red Test is positive if a red coloration develops immediately. A negative reaction is indicated by a yellow color on the surface of the liquid medium.

VP test is positive when a pink to bright red coloration develops 15 minutes after the addition of reagents.

**STORAGE**

10-25°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

**WARNING AND PRECAUTIONS**

The product is not classified as hazardous by current legislation and does not contain harmful substances in concentrations of  $\geq 1\%$ . The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

**DISPOSAL of WASTE**

Disposal of waste must be carried out according to the national and local regulations in force.

**REFERENCES**

1. Voges and Proskauer. 1898. Z. Hyg. 28:20.
2. Clark and Lubs. 1915. J. Infect. Dis. 17:160.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore, Md.
4. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di De Medici, L. Fenicia, L. Orefice e A. Stacchini.

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**PRODUCT SPECIFICATION**

**NAME**

**MR- VP BROTH**

**PRESENTATION**

Glass tubes containing 10 mL of medium.

**STORAGE**

10-25°C

**PACKAGING**

Code	Content	Packaging
24149	20 tubes x10 ml	20 tubes in cardboard box

**pH OF THE MEDIUM**

7.0 ± 0.2

**USE**

**MR-VP BROTH** is a medium for Gram-negative bacteria differentiation based on the Methyl Red and Voges- Proskauer tests, recommended by ISO 6785: 2001.

**TECHNIQUE**

Refer to technical sheet of the product.

**APPEARANCE OF THE MEDIUM**

Appearance: clear to slightly hazy.

Colour: yellow to tan.

**SHELF LIFE**

2 years

**QUALITY CONTROL**

- Control of general characteristics, label and print
- Sterility control  
 7 days at 25 ± 1°C, in aerobiosis  
 7 days at 36 ± 1°C, in aerobiosis
- Microbiological control  
 Inoculum for productivity: 10-100 UFC/ml  
 Inoculum for specificity: ≤ 10<sup>4</sup> UFC/ml  
 Incubation Conditions: 24-48 h at 36 ± 1°C

Microorganisms		Growth	MR Test	VP Test
<i>Escherichia coli</i>	ATCC 25922	good	+	-
<i>Klebsiella pneumoniae</i>	ATCC 13883	good	-	+
<i>Enterobacter aerogenes</i>	ATCC 13048	good	-	+

**TABLE of SYMBOLS**

<b>IVD</b> In vitro Diagnostic Medical Device	<b>LOT</b> Batch code	Manufacturer	Contains sufficient for <n> tests
<b>REF</b> Catalogue number	Temperature limitation	Use by	Caution, consult accompanying documents



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