# OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

# EOSIN METHYLENE BLUE AGAR (LEVINE) (CM0069)

**EOSIN METHYLENE BLUE AGAR (LEVINE)** **CM0069**

**Typical Formula***

| | | |
|---|---|---|
| Peptone | grams per litre | 10.0 |
| Lactose | | 10.0 |
| Di-potassium hydrogen phosphate | | 2.0 |
| Eosin Y | | 0.4 |
| Methylene blue | | 0.06 |
| Agar | | 15.0 |

* adjusted as required to meet performance standards

**Directions**

Suspend 37.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well to ensure even dispersion of the medium in order to oxidize the methylene blue, which is an essential part of this medium, and pour into sterile Petri dishes.

**Physical Characteristics**

Purple, grey or pink free-flowing powder
Colour on reconstitution - dark purple
Moisture level - less than 7%
pH 6.8 ± 0.2 at 25°C
Clarity - clear
Gel strength - firm, comparable to 15.0g/litre of agar

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**Microbiological Tests Using Optimum Inoculum Dilution**

Control Media: Tryptone Soya Agar or Sabouraud Dextrose Agar, as appropriate

**Reactions after incubation at 37°C for 24 hours**

Medium is challenged with 10-100 colony-forming units

| | | |
|---|---|---|
| *Escherichia coli* | ATCC® 10536 | 0.5-2mm purple colonies, with or without metallic green sheen |
| *Escherichia coli* | ATCC® 8739 | 0.5-2mm purple colonies, with or without metallic green sheen |
| *Enterobacter aerogenes* | ATCC® 13048 | 1-2mm purple, mucoid colonies, no sheen |
| *Pseudomonas aeruginosa* | ATCC® 9027 | 0.5-3mm translucent, colourless colonies |

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Some strains of *Escherichia coli* may produce little or no sheen with single colonies.

*Escherichia coli* are also tested using high inocula.

**Reactions after incubation at 37°C for 24 hours**

Medium is challenged with 1E+04 to 1E+06 colony-forming units

Inoculation using diminishing sweep technique

| | | |
|---|---|---|
| *Escherichia coli* | ATCC® 10536 | 0.5-2mm purple, green metallic colonies |
| *Escherichia coli* | ATCC® 8739 | 0.5-2mm purple, green metallic colonies |
| *Escherichia coli* | ATCC® 25922 | 0.5-2mm purple, green metallic colonies |

Inoculation using diminishing sweep technique

| | | |
|---|---|---|
| *Staphylococcus aureus* | ATCC® 6538 | No growth to confluent micro-colonies, no sheen |
| *Staphylococcus aureus* | ATCC® 25923 | No growth to confluent micro-colonies, no sheen |

Negative strains are inhibited or shall produce confluent micro-colonies.

**Reactions after incubation at 37°C for 48 hours 10% $CO_2$ atmosphere**
**(for details, refer to Oxoid Manual - Atmosphere Generation Systems)**

Medium is challenged with 10-100 colony-forming units

| | | |
|---|---|---|
| *Candida albicans* | ATCC® 10231 | 0.25-1mm grey, feather-edged colonies |

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

Equivalent results are obtained after incubation at 35°C for 48 hours in 10% $CO_2$ atmosphere.

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**Tested in accordance with current CLSI M22 A**

Medium is challenged with 10-100 colony-forming units

| | | |
|---|---|---|
| *Escherichia coli* | ATCC® 25922 | 0.5-2mm purple, green metallic colonies |
| *Salmonella typhimurium* | ATCC® 14028 | 0.5-1mm grey, translucent colonies |
| *Enterococcus faecalis* | ATCC® 29212 | Pinpoint, colourless colonies with no sheen |

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

For *Enterococcus faecalis* ATCC®29212, a satisfactory result is represented by recovery of positive strains equal to or greater than 40% of the control medium.

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## Revision History

| Section / Step | Description of Change | Reason for Change | Reference |
|---|---|---|---|
| Entire Document | Addition of CLSI specification | Change control | BT-CC-1502 |