



## Minimal Agar

M512

Minimal Agar is recommended for the isolation and characterization of nutritional mutants of *Escherichia coli*.

### Composition\*\*

Ingredients	Gms / Litre
Dextrose	1.000
Dipotassium phosphate	7.000
Monopotassium phosphate	2.000
Sodium citrate	0.500
Magnesium sulphate	0.100
Ammonium sulphate	1.000
Agar	15.000
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 26.6 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Nutritional mutants of *Escherichia coli* obtained by the exposure of wild type *E. coli* to ultra violet light need a nutritionally complete medium to grow. Minimal media can be supplemented with the desired additives to study nutritional characters of the nutritional mutants. Minimal media are the formulations of Davis (1) as described by Lederberg (2). Minimal media contain the necessary nutrients only for the growth of wild type *E. coli* strains. By the random isolation method described by Lederberg, nutritional mutants derived from irradiated cultures of wild type *E. coli* can be isolated (2). These mutants can also be isolated by the use of penicillin as described by Davis and Lederberg (1). *Bacillus subtilis* mutants can be isolated by these techniques and by the penicillin technique also, as described by Nester et al (3).

Dextrose is an energy source. Dipotassium and monopotassium phosphates provide buffering to the medium. Magnesium sulphate and ammonium sulphate are sources of ions that simulate metabolism.

The nutritional supplements to be added to minimal medium depend upon the type of mutant to be screened as for amino acids, vitamins, nucleic acids or other substances. This can be achieved by addition of vitamin assay casamino acids plus tryptophan or a mixture of water soluble vitamins, yeast or nucleic acid extracts.

A cell suspension of wild type *E. coli* is irradiated and cultured on Minimal Agar supplement with all the necessary growth requirements. This will allow growth of both wild type cells (prototrophs) and mutant cells. The selected colonies are then added to Minimal Broth, Davis (M389) and Minimal Broth Davis supplemented with the growth requirements and incubated at 35°C for 24 hours. Growth in the Minimal Broth supplemented with growth requirements and no growth in Minimal Broth indicates a mutant for that particular factor.

### Quality Control

#### Appearance

Off-white to beige homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

M512: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

<b>Organism</b>	<b>Inoculum (CFU)</b>	<b>Growth</b>	<b>Recovery</b>
<i>Escherichia coli</i> ATCC 13762	50-100	luxuriant	>=50%
<i>Escherichia coli</i> ATCC 23724	50-100	luxuriant	>=50%

**Storage and Shelf Life**

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

**Reference**

1. Davis B. D., 1949, Proc. Natl Acad. Sci, 35:1.
2. Lederberg J., 1950, Methods in Med. Res., 3:5.
3. Nester E. W., Schafer M. and Lederberg J., 1963, Genetics, 48:529.

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