TM 591 - MINIMAL AGAR

INTENDED USE

For isolation and characterization of nutritional mutants of *Escherichia coli*.

PRODUCT SUMMARY AND EXPLANATION

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 as described by Lederberg. 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. These mutants can also be isolated by the use of penicillin as described by Davis and Lederberg. *Bacillus subtilis* mutants can be isolated by these techniques and by the penicillin technique also, as described by Nester et al.

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 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

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.

INSTRUCTION FOR USE

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

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Off-white to beige homogeneous free flowing powder.
Appearance of prepared medium : Medium amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.0±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	13762	50-100	Luxuriant	>=70 %	35-37°C	18-24 Hours
Escherichia coli	23724	50-100	Luxuriant	>=70 %	35-37°C	18-24 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

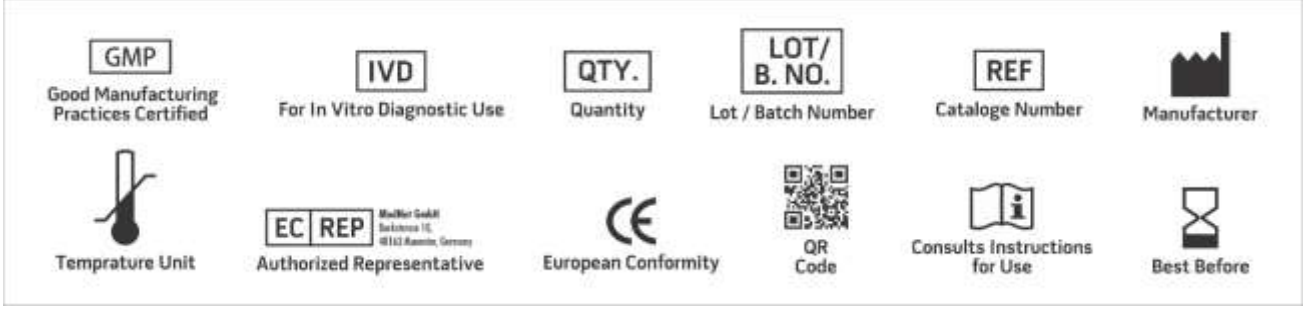
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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