Minimal Agar Davis
Minimal Broth Davis without Dextrose

Intended Use

Minimal Agar Davis is used for isolating and characterizing nutritional mutants of *Escherichia coli*.

Minimal Broth Davis without Dextrose is used with added dextrose in isolating and characterizing nutritional mutants of *Escherichia coli* and *Bacillus subtilis*.

Summary and Explanation

Lederberg[1] described the Davis formulation for Minimal Agar Davis. Minimal Broth Davis without Dextrose is the same formulation without dextrose and agar. Both media support the growth of nutritional mutants of *E. coli* while Minimal Broth Davis without Dextrose with added dextrose also supports the growth of nutritional mutants of *B. subtilis*.

Lederberg[1] described two techniques for isolating nutritional mutants of *E. coli*, one by random isolation and the other by delayed enrichment. Both Lederberg[1] and Davis[2] described a third technique using penicillin. Nutritional mutants of *B. subtilis* can be isolated by these three techniques and by a modification of the penicillin technique described by Nester, Schafer and Lederberg.[3]

After the mutants are isolated, they are characterized biochemically by growth in minimal broth supplemented with specific growth factors or groups of growth factors. It is generally best to classify mutants according to their requirements for amino acids, vitamins, nucleic acids or other substances. This is done by supplementing the minimal medium with Vitamin Assay Casamino Acids plus tryptophan, or a mixture of water soluble vitamins, alkaline-hydrolyzed yeast, nucleic acid or yeast extract, depending on the particular mutants desired. The supplemented minimal broth is inoculated with a slightly turbid suspension of the mutant colonies and incubated for 24 hours at 35°C. Growth with Vitamin Assay Casamino Acids indicates a vitamin requirement. When a major growth factor group response is obtained, the characterization is carried further by the same general procedure to subgroups and finally to individual growth substances.

Principles of the Procedure

Minimal Agar Davis and Minimal Broth Davis without Dextrose contain citrate and phosphates as buffers. Ammonium sulfate is the nitrogen source. Magnesium is a cofactor for many metabolic reactions. Minimal Agar Davis contains dextrose as the carbohydrate energy source. Agar is the solidifying agent.

Formulae

Difco™ Minimal Agar Davis

Approximate Formula* Per Liter

Dextrose	1.0	g
Dipotassium Phosphate	7.0	g
Monopotassium Phosphate	2.0	g
Sodium Citrate	0.5	g
Magnesium Sulfate	0.1	g
Ammonium Sulfate	1.0	g
Agar	15.0	g

Difco™ Minimal Broth Davis without Dextrose

Consists of the same ingredients without the dextrose and agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Minimal Agar Davis

1. Suspend 26.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Minimal Broth Davis without Dextrose

1. Dissolve 10.6 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. If desired, aseptically add 10 mL of 10% dextrose solution at room temperature. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Random Technique

1. Irradiate a cell suspension of wild type *E. coli*.
2. Dilute the suspension 100-500×.
3. Culture on a complete agar medium containing all the necessary growth requirements.
4. Incubate the cultures at 35 ± 2°C for 24 hours.
5. Select isolated colonies and inoculate into Minimal Broth Davis and a nutritionally complete broth.
6. Incubate at 35 ± 2°C for 24 hours.
7. Observe growth in both media.

User Quality Control

Identity Specifications

Difco™ Minimal Agar Davis

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in purified water upon boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 2.66% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ Minimal Broth Davis without Dextrose

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	1.06% solution, soluble in purified water. Solution is colorless, clear.
Prepared Appearance:	Colorless, clear.
Reaction of 1.06% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Minimal Agar Davis

Prepare the medium per label directions. Inoculate by the pour plate method and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	6883	10^2-10^3	Good
Escherichia coli	9637	10^2-10^3	Good

Difco™ Minimal Broth Davis without Dextrose

Prepare the medium per label directions with the addition of 1% dextrose. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10^2-10^3	Good
Escherichia coli	6883	10^2-10^3	Good
Escherichia coli	9637	10^2-10^3	Good

Delayed Enrichment Method

1. Prepare plates of Minimal Agar Davis by pouring a 15-20 mL base layer in a 95 mm sterile Petri dish followed by a 5 mL seed layer.
2. Inoculate with a diluted irradiated *E. coli* suspension.
3. Pour a 5-10 mL layer of uninoculated Minimal Agar Davis over the seed layer.
4. Incubate for 24 hours or longer to allow for the growth of prototroph cells (wild type cells).
5. Pour a layer of a complete agar medium over the minimal agar medium to develop the mutant cells.
6. Incubate at 35 ± 2°C for 6-12 hours.

Penicillin Method

1. Wash an irradiated *E. coli* suspension with sterile saline and dilute to 20 × the original volume in sterile minimal broth.
2. Dispense into tubes in desired amounts.
3. Add freshly prepared penicillin to each tube to give a final concentration of 200 units per mL.
4. Incubate at 35 ± 2°C for 4-24 hours on a shaker.
5. Spread 0.1 mL, 0.01 mL and 0.001 mL samples onto complete agar plates.
6. Incubate at 35 ± 2°C for 24 hours.
7. Select isolated colonies and test for growth in minimal broth.

Bacillus subtilis Method

1. Grow cultures of *Bacillus subtilis* in Antibiotic Medium 3 at 35 ± 2°C for 18 hours.
2. Centrifuge to sediment the cells.
3. Aseptically decant the supernatant fluid.
4. Resuspend the cells in minimal medium and centrifuge.
5. Decant the supernatant and resuspend the pellet in minimal medium to give a cell concentration of about 2×10^8 cells per mL.
6. Irradiate the suspension with a low pressure mercury ultraviolet lamp for a sufficient time to give a cell survival of 1×10^4 cells per mL.
7. Incubate the suspension at room temperature for 4-18 hours in the minimal medium with appropriate substances added to allow for the growth of desired mutants.
8. Wash the culture in sterile minimal medium.
9. Centrifuge and resuspend in the same medium.
10. Dilute 1 to 10 with sterile minimal medium.
11. Let stand for 60 minutes to starve the mutants.
12. Add penicillin to give a concentration of 2,000 units per mL.
13. Incubate 15 minutes.
14. Plate the culture on nutrient agar for colony isolation.
15. Identify the nutrition mutants by transferring colonies by replicate plating onto plates of minimal agar which has been supplemented with the appropriate nutritional substances.

Expected Results

Random Technique

Growth in the nutritionally complete medium and no growth in the Minimal Broth indicates a mutant.

Delayed Enrichment Method

Mutant colonies will grow as small colonies after the addition of the complete medium which diffuses through the Minimal Agar.

Penicillin Method

Mutant colonies grow after the addition of penicillin.

Bacillus subtilis Method

Mutant colonies grow on Nutrient Agar after the addition of penicillin.

Limitation of the Procedure

Strains vary in their sensitivity to penicillin. Adjustments to the time of treatment and concentration of penicillin may be necessary.[1]

References

1. Lederberg. 1950. Methods in Med. Res. *3*:5.
2. Davis. 1949. Proc. Natl. Acad. Sci. *35*:1.
3. Nester, Schafer and Lederberg. 1963. Genetics *48*:529.

Availability

Difco™ Minimal Agar Davis

Cat. No. 254410 Dehydrated – 500 g

Difco™ Minimal Broth Davis without Dextrose

Cat. No. 275610 Dehydrated – 500 g