M9 Minimal Salts, 5×

Intended Use

M9 Minimal Salts, 5× is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9 Minimal Salts, 5× is a 5× concentrate that is diluted to a 1× concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium. The medium will support the growth of "wild-type" strains of *E. coli*. M9 Minimal Salts is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F′-containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs.

Principles of the Procedure

Sodium phosphate and potassium phosphate are present as buffering agents. Ammonium chloride is a source of nitrogen for cellular systems. Sodium chloride provides essential ions. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Formula

Difco™ M9 Minimal Salts, 5×

Approximate Formula* Per Liter		
Disodium Phosphate (anhydrous)	33.9	g
Monopotassium Phosphate	15.0	g
Sodium Chloride	2.5	g
Ammonium Chloride	5.0	g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 56.4 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. To prepare M9 Minimal Salts Medium, add 200 mL sterile M9 Minimal Salts, 5× to 750 mL sterile purified water cooled to 45-50°C, adjusting the final volume to 1 liter.
4. Aseptically add 20 mL filter-sterilized 20% glucose solution, 2 mL sterile 1.0 M $MgSO_4$ solution and, if desired, 0.1 mL sterile 1.0 M $CaCl_2$ solution. Mix well.
5. If desired, supplement with amino acids, as appropriate.
6. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ M9 Minimal Salts, 5×

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in purified water. Solution is colorless, clear, no significant precipitate.
Prepared Appearance:	Colorless, clear, no siginficant precipitate.
Reaction of 5.64% Solution (5× concentrate) at 25°C:	pH 6.8 ± 0.2

Cultural Response

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Prepare the medium and dilute to 1x. Supplement with glucose per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli (Strain B)	23226	30-300	Good to excellent
Escherichia coli (JM103)	39403	30-300	Good to excellent

Procedure

Consult appropriate references for recommended test procedures.[1,2]

Expected Results

Growth should be evident by the appearance of turbidity.

References

1. Davis, Dibner and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Availability

Difco™ M9 Minimal Salts, 5×

Cat. No. 248510 Dehydrated – 500 g