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 MgSO₄ solution, and, if desired, 0.1 mL sterile 1.0 M CaCl₂ 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.

Procedure
Consult appropriate references for recommended test procedures.¹²

Expected Results
Growth should be evident by the appearance of turbidity.

References

Availability
*Difco™ M9 Minimal Salts, 5×*
Cat. No. 248510 Dehydrated – 500 g