

Technical Information

Hanahans Broth (SOB Medium)

Product Code: DM 2252

Application: - Hanahans Broth is recommended for use in cultivation of recombinant strains of *Escherichia coli*.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Yeast extract	5.000
Sodium chloride	0.500
Magnesium sulphate	2.400
Potassium chloride	0.186
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Transformation is a process involving the uptake of foreign genetic material, The ability of a bacterium to take up exogenous DNA from the extracellular environment is termed as competence of the bacterium which on subsequent recombination event results into genetically altered cell. Factors affecting the cell surface particularly changes in the membrane permeability, are important so as to allow the foreign DNA to enter the recipient cell ⁽¹⁾. Bacteria undergoing transformation need to be cultured on a rich, isotonic medium so that it can overcome or recover from the process of transformation by mending the perforations caused by transformation and undergo replication ⁽²⁾. Hanahans Broth developed by Hanahan ⁽³⁾ is used for the cultivation of these recombinant *Escherichia coli* strains that have undergone transformation.

Hanahans Broth is a nutritionally rich growth medium used in the preparation and transformation of competent cells. For generation of competent cells, the bacteria is grown in Hanahans Broth to get desired turbidity and subjected to standard procedures such as electroporation or treatment with CaCl₂ in chilled conditions. For the survival of such perforated, competent cells, a rich, isotonic environment is needed. Hanahans Broth with 0.4% dextrose is used in the final stage of transformation, which provides carbon and energy source for mending the perforations and subsequent replication ⁽²⁾.

Casein enzymic hydrolysate and yeast extract in the medium supply nitrogenous compounds and growth factors for the recombinant *E. coli*. Potassium and sodium chloride maintains isotonic conditions. Magnesium sulphate is added to the medium as the necessary component for DNA replication.

Methodology

Suspend 28.08 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear solution without any precipitate

Reaction

Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH Range 6.80-7.20

Cultural Response/Characteristics

DM 2252: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
<i>Escherichia coli</i> DH5 53868	50-100	good-luxuriant

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

1. Dundas I.E., 1977, Advances In Microbiology and Physiology, Rose H. and Tempest D.W. (Eds.), A.P. London.
2. Gibbons N.E., 1969, Methods In Microbiology, Vol. 3B, Norris J.R., and Ribbons D.W. (Eds.), A.P., New York, pp.169-183.
3. Kushner D. J., (Eds.), 1978, D. J. Kushner, pg 317, Academic Press, London, England
4. MacLeod R. A., 1965, Bacteriol., Rev., 29:9

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