



## Hanahans Broth (SOB Medium)

M1252

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

### Directions

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.

### Principle And Interpretation

Transformation is a process involving the uptake of foreign genetic material, which on subsequent recombination event results into genetically altered cell. The ability of a bacterium to take up exogenous DNA from the extracellular environment is termed as competence of the bacterium. Factors affecting the cell surface are important to competence, particularly changes in the membrane permeability, 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 to 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 for use in the preparation and transformation of competent cells. For generation of competent cells, the bacteria is grown in Hanahans Broth to the desired turbidity and subjected to standard procedures such as electroporation or treatment with CaCl<sub>2</sub> in chilled conditions to achieve competence. 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.

### Quality Control

#### 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

6.80-7.20

#### Cultural Response

M1252: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
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#### Cultural Response

*Escherichia coli* DH5 53868 50-100      good-luxuriant

### Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

### Reference

1. Alcamo I. E., 2001, Fundamentals of Microbiology, 6th Edition, Jones and Bartlett Publishers.
2. Sambrook J., Fritsch E. E. and Maniatis T., 1989, Molecular Cloning : A Laboratory Manual, 2nd Ed., Cold Spring Harbor Lab. Press; Cold Spring Harbor, N.Y.
3. Hanahan D., 1983, J. Mol. Biol., 166:557.

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