



PRODUCT SPECIFICATION SHEET

Hanahan's Broth (SOB medium) (DM581)

Intended Use

Hanahan's Broth (SOB medium) (DM581) is recommended for cultivation of recombinant strains of *Escherichia coli*.

Product Summary and Explanation

Transformation is a process involving the uptake of foreign genetic material, which on subsequent recombination event results into genetically altered cell. Transformation requires making perforations in the bacterium (i.e., making the cells "competent") to allow the introduction of foreign DNA into the cell. Competence is the ability of a bacterium to take up exogenous DNA from the extracellular environment. 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⁾ To survive transformation process bacteria 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_2 in chilled conditions to achieve competence. 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. This addition provides a readily available source of carbon and energy in a form *E. coli* can use in mending the perforations and for replication.⁽²⁾

Principles of the Procedure

Hanahan's Broth contains casein enzymic hydrolysate yeast extract provide sources of nitrogen and growth factors which allow the bacteria to recover from the stress of transformation and grow well. Potassium and sodium chloride maintains isotonic conditions. Magnesium sulphate is added to the medium as the necessary component for DNA replication.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Yeast extract	5.00
Sodium chloride	0.50
Magnesium sulphate	2.40
Potassium chloride	0.186
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 28.08 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in tubes. Autoclave at 121°C , 15 psi pressure, for 15 minutes / validated cycle.





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Quality Control Specifications

Dehydrated Appearance	Cream yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured, clear solution without any precipitate
Reaction of 2.8% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Escherichia coli</i> DH5 53868	50-100	good-luxuriant

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer appropriate references for standard test procedures.

Results

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Hanahan's Broth (SOB medium)

Product Code : DM581

Available Pack sizes : 500gm

References

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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Further Information

For further information please contact your local MICROMASTER Representative.



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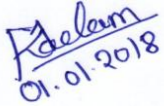
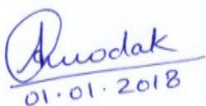

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Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) - 400607. M.S. INDIA.

Ph: +91-22-25895505, 4760, Cell: 9320126789.

Email: micromaster@micromasterlab.com

sales@micromasterlab.com

Prepared By	Checked By	Approved By
 01.01.2018	 01.01.2018	 01.01.2018
Microbiologist	Head Quality Control	Head Quality Assurance

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