



## PRODUCT SPECIFICATION SHEET

### Clausen Medium (DM061)

#### Intended Use

Clausen Medium (DM061) is recommended for sterility testing in compliance with Nordic Pharmacopoeia Board.

#### Product Summary and Explanation

Clausen Medium also called Dithionite-thioglycollate (HS-T) Broth was developed by Clausen<sup>(1)</sup> and has been recommended for sterility testing by the Nordic Pharmacopoeia Board. The problem of sterility testing by selecting random samples is recognised by the Board and they refer to the process as the Microbial-Contamination Test. The standard microbial-contamination test is designed solely to establish that the number of non-sterile units, if any, in a batch is below a certain level. Random sampling in sufficient quantity of the bulk should be examined.

#### Principles of the Procedure

Clausen Medium contains enzymic hydrolysate, papaic digest of soyabean meal which provides nitrogen, carbon, and amino acids essential growth nutrients. Yeast extract is a rich source of vitamin B complex. Dextrose is an energy source for the metabolism of fungi. Sodium chloride helps to maintain the osmotic balance of the medium. L-cystine and sodium thioglycollate act as reducing agents, and the essential metals help for isolating anaerobic sporeformers. Polysorbate 80 and lecithin are added in this medium to overcome the effects of cationic agents, which can exert bacteriostatic effect in vitro. This medium is clear in appearance and yellow coloured. Under aerobic conditions it turns pink. Therefore at the time of use the upper one third of the medium should be pink.

#### Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	3.00
Yeast extract	6.00
Dextrose	6.00
Sodium chloride	2.50
Dipotassium phosphate	2.00
Sodium citrate	1.00
L-Cystine	0.50
L-Asparagine	1.25
Sodium dithionite	0.40
Sodium thioglycollate	0.50
Lecithin	0.30
Magnesium sulphate	0.40
Calcium chloride	0.04
Cobalt sulphate	0.001
Cupric sulphate	0.001
Ferrous sulphate	0.001
Zinc sulphate	0.001
Manganese chloride	0.002
Resazurin	0.001
Agar	0.75
Final pH: 7.1 ± 0.2 at 25°C	





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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.
3. If more than upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink colour disappears.

### Directions

1. Suspend 40 grams of the medium in one liter of distilled water containing 3 grams polysorbate 80 and 5 grams glycerol.
2. Heat to boiling to dissolve the medium completely.
3. Dispense as desired.
4. Autoclave at 118°C, 15 psi pressure, for 15 minutes.
5. Place in cool dark place till use.
6. DO NOT RESTERILIZE the medium.

### Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light straw coloured, clear to slightly opalescent solution with upper 10% or less portion pink on standing
Reaction of 4% containing 0.3% polysorbate 80 and 0.5% glycerol	pH 7.1 ± 0.2 at 25°C
Gel Strength	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant
2.	<i>Candida albicans</i> ATCC 10231	50-100	good-luxuriant
3.	<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant
4.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	good-luxuriant
5.	<i>Staphylococcus aureus</i> ATCC 25923	50-100	good-luxuriant
6.	<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant
7.	<i>Streptococcus pyogenes</i> ATCC 19615	50-100	good-luxuriant

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

### Test Procedure

#### Membrane Filter Method

1. The test substance is dissolved or suspended in 200ml of 0.1% w/v sterile (pH 7.0±7.2) Peptone water (DM192) and immediately filtered through one or more membrane filters (average pore diameter 0.45µm or less).
2. Each filter is then washed three times, by passing 100ml volumes of peptone solution through the membrane.
3. After filtration the membranes are transferred to tubes of media, containing at least 15ml of Clausen Medium and tubes of Tryptone Soya Broth (DM277).
4. If only one filter is used, this is divided into two and the two halves placed in separate tubes.





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5. Tubes of Clausen Medium are incubated for at least 14 days at  $30\pm 32^{\circ}\text{C}$ . Tubes of Tryptone Soya Broth are incubated for at least 14 days at  $20\pm 25^{\circ}\text{C}$ .

### Dilution Method

1. From each sample 1.0ml of material or suspension is transferred to each of at least 10 tubes containing a minimum quantity of 15ml of Clausen Medium.
  2. One half of the number of tubes is incubated at  $30\pm 32^{\circ}\text{C}$  for at least 14 days and the other half at  $20\pm 25^{\circ}\text{C}$  for the same time.
  3. If the medium becomes turbid on incubation, subcultures must be taken as soon as possible.
  4. Subcultures must also be taken after normal incubation and observed for a further period of 14 days.
- Refer to appropriate references for standard test procedures.

### Results

1. The standard microbial contamination test is passed if growth is not observed in any of the tubes.
2. Growth is diagnosed by the appearance of turbidity in fluid or semi-fluid media, by the formation of colonies on solid media, or by microscopy of culture samples.
3. Refer to references and test procedures for interpretation of results.

### Storage

Store the sealed bottle containing the dehydrated medium at  $10 - 30^{\circ}\text{C}$ . 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 : Clausen Medium

Product Code : DM061

Available Pack sizes : 500gm

### References

1. Clausen O.G., 1973, Pharmaceutica Acta Helvetiae, 48:541. Further Information  
For further information please contact your local MICROMASTER Representative.



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