# PRODUCT SPECIFICATION SHEET

## Brain Heart Infusion Agar (DM810)

**Intended Use**

Brain Heart Infusion Agar is an enriched non-selective medium used for cultivation of wide variety of fastidious pathogenic bacteria, yeasts and molds. With the addition of 5% or 10% sheep blood, it is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi, from clinical and nonclinical sources.

**Product Summary and Explanation**

Rosenow described brain-heart infusion Agar prepared by adding pieces of brain tissue to meat infusion or beef extract dextrose broth.[1] In the early years of bacteriology, meat infusions were utilized as the growth-supporting components in a large number of culture media. Although, they were cumbersome to prepare, lacked consistency from batch to batch and were undefined as to their nutritive content, they enabled the cultivation of microorganisms in both solid and liquid media. Advanced enzymology and chemistry, lead to development of methods for the preparation of peptones that were the result of enzymatic or acid hydrolysis of animal tissues or products and vegetable substances. These peptones currently are the major nutritional additives to culture media formulations, but infusions are still utilized in specific media. Hayden modified the original formula while working with dental pathogens.[2] The current formula is thus, a modification of Rosenow and Hayden, using dehydrated infusions of calf brain and beef heart tissue.[1,2] Brain Heart Infusion Agar is highly nutritious and can support luxuriant growth of wide variety of microorganisms. It can be further enriched by the addition of blood or rendered selective by adding different antibiotics. [1, 2] It is a general purpose medium used for primary isolation of aerobic bacteria from clinical specimens. Brain Heart Infusion Agar with 10% Sheep Blood can be used to isolate systemic fungi that may grow poorly on the non-enriched medium. [3, 4, 5] Antimicrobial agents, including chloramphenicol, gentamicin, and penicillin in combination with streptomycin, can be incorporated to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria. [6]

**Principles of the Procedure**

Brain Heart Infusion Agar is nutritious and well buffered medium to support the growth of wide variety of organisms. Proteose peptone and infusions (calf brain and beef heart) serve as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins to support the growth of fastidious and non fastidious organisms. Dextrose is the carbon energy source. Disodium phosphate acts as a buffering agent whereas; sodium chloride acts as a differential and/or selective agent by interfering with membrane permeability and maintains the osmotic balance of the medium. Defibrinated sheep blood added to the basal medium provides essential growth factors for the more fastidious fungal organisms.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Calf brain infusion from | 200.00 |
| Beef heart infusion from | 250.00 |
| Proteose peptone | 10.00 |
| Dextrose | 2.00 |
| Sodium chloride | 5.00 |
| Disodium phosphate | 2.50 |
| Agar | 15.00 |
| Final pH: 7.4 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

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**Precautions**

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

**Directions**

1. Suspend 52 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well before pouring.
5. If desired, 20 units Penicillin and 40 µg Streptomycin per ml of medium may be added to make the medium selective for fungi.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Cream to yellow colored, homogeneous, free flowing powder |
| **Prepared Medium** | Basal medium : Light amber colored, clear to slightly opalescent gel<br>After addition of 5% v/v sterile defibrinated blood : Cherry red colored, opaque gel forms in the Petri plates. |
| **Reaction of 3.7% Solution** | pH : 7.4 ± 0.2 at 25°C |
| **Gel Strength** | Firm, comparable with 1.5% Agar gel |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (If desired add 5% v/v sterile defibrinated blood).

| Sr. No. | Organisms | Results to be achieved | | | | |
|---|---|---|---|---|---|---|
| | | Inoculum (CFU) | Growth | Recovery | Growth w/blood | Recovery w/blood |
| 1. | *Candida albicans ATCC 26790* | 50 -100 | good-luxuriant | >=70% | good-luxuriant | >=70% |
| 2. | *Escherichia coli ATCC 25922* | 50 -100 | good-luxuriant | >=70% | good-luxuriant | >=70% |
| 3. | *Shigella flexneri ATCC 12022* | 50 -100 | good-luxuriant | >=70% | good-luxuriant | >=70% |
| 4. | *Staphylococcus aureus ATCC 25923* | 50-100 | good-luxuriant | >=70% | good-luxuriant | >=70% |
| 5. | *Streptococcus pneumonia ATCC 6303* | 50-100 | good-luxuriant | >=70% | good-luxuriant | >=70% |

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

**Test Procedure**

1. Additives (e.g., blood) can be used as desired while pouring the liquefied medium into sterile plates.
2. To obtain isolated colonies from specimens, use standard procedures.
3. Since many pathogens require carbon dioxide on primary isolation, plates of plain Brain Heart Infusion Agar may be incubated in an atmosphere containing approximately 5-10% $CO_2$.
4. Incubate plates at 35 - 37°C for 24-48 hours.
5. For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the non-selective medium.

6. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 - 37°C.
7. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.
8. Brain Heart Infusion Agar slants primarily are used for the cultivation and maintenance of pure cultures of microorganisms.

**Results**

1. After sufficient incubation, isolated colonies are observed on the plates, in the streaked areas and heavy inoculation areas show confluent growth.
2. When culturing for fungi, examine plates for fungal colonies exhibiting typical color and morphology.
3. Biochemical tests and serological procedures should be performed to confirm findings.
4. Slant cultures may be used as sources of inocula for additional studies or for organism maintenance purposes.

**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.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

**Packaging**

**Product Name : Brain Heart Infusion Agar.**
**Product Code : DM810**
**Available Pack sizes : 100gm / 500gm**

**References**

1. Rosenow, E. C. 1919. Studies on elective localization. J. Dent. Research 1:205-249.
2. Hayden, R. L. 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. 32:828-849.
3. Creitz and Puckett, 1954, Am. J. Clin. Pathol., 24:1318.
4. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
5. Ajello L., Georg L., Kaplan W. and Kaufman L., 1963, CDC Laboratory Manual for Medical Mycology, PHS Publication
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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**Further Information**

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