

Technical Information

Reinforced Clostridia Broth

Product Code: DM 1443

Application: Rhizobium Medium is used for cultivation and isolation of *Rhizobium* species.

| Ingredients | Gms / Litre | |
|--|-------------|--|
| Casein enzymic hydrolysate | 10.000 | |
| Beef extract | 10.000 | |
| Yeast extract | 3.000 | |
| Dextrose | 5.000 | |
| Sodium chloride | 5.000 | |
| Starch, soluble | 1.000 | |
| L-Cysteine hydrochloride | 0.500 | |
| Sodium acetate | 3.000 | |
| Agar | 0.500 | |
| Final pH (at 25°C) **Formula adjusted, standardized to suit performance parameters | 6.8±0.2 | |

Principle & Interpretation

Reinforced Clostridial Broth has been devised by Hirsch and Grinsted⁽¹⁾. It can be used with small inocula to obtain the highest viable count of *Clostridia*. Barnes and Ingram⁽²⁾ used the broth medium for diluting inoculum of vegetative cells of *Clostridium perfringens*⁽²⁾. It can also be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese, for enumeration of Clostridia in tube dilution counts or for preparation of plates for isolation⁽³⁾. Other spore forming anaerobes, *Streptococci* and *Lactobacilli* also grow in this media. This media is both nonselective & environment nature wise.

Casein enzymic hydrolysate, yeast extracts, beef extract, starch, L-cysteine and sodium acetate provide all the necessary nutrients for the growth of *Clostridia*. Dextrose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. This media can be made selective by addition of 15-20 mg Polymyxin B per litre of media ⁽¹⁾.

Methodology

Suspend 38.00 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes. Mix well and dispense as desired.

Quality Control

Physical Appearance Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution in tubes.

Reaction Reaction of 3.8% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH range: 6.6-7.0 Cultural Response/Characteristics DM 1443: Cultural characteristics observed in an anaerobic atmosphere after an incubation at 35 - 37°C for 40 - 48 hours.





Dehydrated Culture Media Bases / Media Supplements

| Organism | Inoculum (CFU) | Growth |
|--------------------------------------|-------------------|------------------|
| Clostridium sporogenes ATCC 11437 | 50 -100 | good - luxuriant |
| Clostridium sporogenes ATCC 19404 | 50 -100 | good - luxuriant |
| Bacteroides vulgates ATCC 8482 | 50 -100 | good - luxuriant |
| Bacteroides fragilis ATCC 23745 | 50 -100 | good - luxuriant |
| Clostridium sporogenes ATCC 13124 | 50 -100 | good - luxuriant |

Storage and Shelf Lifez

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. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.

2. Barnes and Ingram, 1956, J. Appl. Bact., 19:117.

3. Lewis and Angelotti (Eds.), 1964, Examination of Foods for Enteropathogenic and Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.

Storage and Shelf Life

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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