

TM 625 – DIFFERENTIAL REINFORCED CLOSTRIDIAL BROTH BASE

INTENDED USE

For cultivation of *Clostridia* from water.

PRODUCT SUMMARY AND EXPLANATION

Hirsch and Grinstead originally described Differential Reinforced Clostridial Medium to initiate the growth from small inoculum and get a higher Clostridial count. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens*. This medium is developed for the isolation of sulphite-reducing *Clostridia* from food and for their enumeration in water by multiple tube method. Differential Reinforced Clostridial Broth is used to determine the count of sulphite reducing bacteria by MPN technique.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	10.000
Beef extract	10.000
Yeast extract	1.500
Starch	1.000
Sodium acetate, hydrated	5.000
Dextrose (Glucose)	1.000
L-Cysteine hydrochloride	0.500

PRINCIPLE

The medium consists of Peptone, Beef extract, yeast extract, starch, sodium acetate which provide essential nutrients for bacterial metabolism. Glucose is the fermentable carbohydrate and serves as carbon and energy source. L-cysteine hydrochloride acts as reducing agent. Sodium sulphite and ferric citrate are added as indicators. Sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black coloured medium.

INSTRUCTION FOR USE

- Dissolve 29 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Just before use add 0.5 ml filter sterilized solution, prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate, to 25 ml of single strength medium or 0.4 ml and 2 ml to 10 ml and 50 ml of double strength medium respectively. Mix well.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Light yellow coloured, clear solution without any precipitate.
pH (at 25°C)	: 7.2 ± 0.2

INTERPRETATION

Cultural characteristics observed in an anaerobic atmosphere, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate after incubation.



Microorganism	ATCC	Inoculum (CFU/ml)	Growth	H ₂ S production	Incubation Temperature	Incubation Period
<i>Clostridium perfringens</i>	13124	50-100	Good-luxuriant	Positive reaction, blackening of medium	30-35°C	1 Week
<i>Clostridium sporogenes</i>	11437	50-100	Good-luxuriant	Positive reaction, blackening of medium	30-35°C	1 Week

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.










Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- 1 Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Hirsch A. and Grinstead E., 1954, J. Dairy Res. 21:101
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Barnes E. M. and Ingram M., 1956, J. Appl. Bacteriol., 19(1):117.
5. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
6. orgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

 GMP Good Manufacturing Practices Certified	 Best Before	 QTY. Quantity	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 LOT/ B. NO. Lot / Batch Number	 Consults Instructions for Use	 QR Code	

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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