# Reinforced Medium for Clostridia (Harmonized)

## Intended Use
Reinforced Medium for Clostridia is used for cultivation and enumeration of Clostridia from pharmaceutical products in accordance with microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

## Summary
Reinforced Medium for Clostridia is formulated by Hirsch and Grinsted. It can be used to initiate growth from small inocula and to obtain the highest viable count of Clostridia. Barnes and Ingrams used the broth medium for diluting an inoculum of vegetative cells of *Clostridium perfringens*. It can 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. Other spore forming anaerobes, Streptococci and Lactobacilli also grow in this media. This is an enriched but non-selective medium.

## Principle
Peptone, yeast extract, beef extract, starch, cysteine hydrochloride and sodium acetate provide all the necessary nutrients for the growth of Clostridia. Glucose is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium.

## Formula*

| **Ingredients** | **g/L** |
|---|---|
| Beef Extract | 10.0 |
| Peptone | 10.0 |
| Yeast Extract | 3.0 |
| Soluble Starch | 1.0 |
| Glucose Monohydrate | 5.0 |
| Cysteine Hydrochloride | 0.5 |
| Sodium Chloride | 5.0 |
| Sodium Acetate | 3.0 |
| Agar | 0.5 |
| Final pH (at 25°C) | 6.8 ± 0.2 |

*Adjusted to suit performance parameters.

## Directions
1. Bring the Reinforced Medium for Clostridia (Harmonized) vial to the room temperature 22°C-30°C.
2. Use Reinforced Medium for Clostridia (Harmonized) as per required application.

## Quality Control
**Appearance:** Light amber coloured, slightly opalescent solution, with fine settlement of agar gel.
**Growth Promotion Test:** Growth promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP and growth is observed after an incubation at 30°C-35°C for 48 hours under anaerobic conditions. Subculturing is carried out using Columbia Agar after enrichment in Reinforced Medium for Clostridia (Harmonized) and incubated at 30°C-35°C for 48-72 hours under anaerobic conditions.
**Growth Promoting Properties:** The test results observed are within the specified temperature and shortest period of time, inoculating 10-100 cfu (at 30°C 35°C for 48 hours, under anaerobic conditions).

| **Organisms (ATCC)** | **Growth** |
|---|---|
| *Clostridium sporogenes* (11437) | Good |
| *Clostridium sporogenes* (19404) | Good |

**Note:** Inoculum for good growth is 10-100 cfu.

## Remarks
1. Do not use media bottles that exhibit any damage, cracks, microbial contamination, discoloration, drying or other sign of deterioration.
2. Ensure that the temperature of water bath is at 100°C so that the medium melts completely. Cooler water baths give rise to lumpy, uneven medium.

3. Before pouring into sterile petriplates, gently swirl the bottle to check whether the entire contents are properly mixed and melted.
4. Good laboratory practices and hazard precautions must be observed at all times.
5. After use media containers, prepared plates, sample, sample containers and other contaminated materials must be sterilized or incinerated before discarding.

## Storage and Stability

1. Store the ready to use Reinforced Medium for Clostridia (Harmonized) at 15°C-25°C in a cool, dry place away from light.
2. Stability of the kit is as per expiry date mentioned on the label.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## References


1. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.
2. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.
6. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.


## Product Presentation:

| Cat. No. | Product Description | Pack Size |
|---|---|---|
| 203180250100 | Bottle Media | 100 mL |