

## Technical Data Sheet

### GranuCult™

### Fluid Thioglycollate Medium

### acc. EP, USP, JP and ISO

Ordering number: 1.08191.0500 / 1.08191.5000

For cultivation and isolation of obligate and facultative anaerobic and microaerophilic bacteria and for sterility tests.

This culture medium complies with the specifications given by the harmonized methods of EP, USP, JP for Biological Tests: Sterility Test and with those given by EN ISO 7937, EN ISO 11133 as well as by FDA-BAM and APHA.

#### Mode of Action

The reducing agents thioglycollate and L-cystine ensure an anaerobiosis which is adequate even for strict anaerobes. The sulfhydryl groups of these substances also inactivate arsenic, mercury and other heavy metal compounds. The thioglycollate medium is thus suitable for the examination of materials which contain heavy metals or heavy metal preservatives.

Glucose, pancreatic digest of casein, L-cystine, yeast extract and sodium chloride provide the growth factors and essential ions. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintain anaerobiosis in the lower depths of the medium.

The higher viscosity of the Fluid Thioglycollate Medium prevents rapid uptake of oxygen. Any increase in the oxygen content is indicated by the redox indicator sodium resazurin which changes its color to red when oxidized.

## Typical Composition

| Specified by EP, USP, JP and ISO 7937 |                 | Specified by BAM M146                       |                  | GranuCult™ Fluid Thioglycollate Medium acc. EP, USP, JP and ISO |           |
|---------------------------------------|-----------------|---------------------------------------------|------------------|-----------------------------------------------------------------|-----------|
| Pancreatic Digest of Casein           | 15 g/l          | Tryptone                                    | 15 g/l           | Pancreatic Digest of Casein*                                    | 15 g/l    |
| Yeast Extract (water-soluble)         | 5 g/l           | Yeast Extract                               | 5 g/l            | Yeast Extract                                                   | 5 g/l     |
| Dextrose Mono-hydrate / Anhydrous     | 5.5 g/l / 5 g/l | Dextrose                                    | 5 g/l            | D(+)-Glucose Monohydrate                                        | 5.5 g/l   |
| L-Cystine                             | 0.5 g/l         | L-Cystine                                   | 0.5 g/l          | L*-Cystine                                                      | 0.5 g/l   |
| NaCl                                  | 2.5 g/l         | NaCl                                        | 2.5 g/l          | NaCl                                                            | 2.5 g/l   |
| Sodium Thioglycollate                 | 0.5 g/l         | Sodium Thioglycollate or Thioglycollic Acid | 0.5 g/l          | Sodium Thioglycollate                                           | 0.5 g/l   |
| Resazurin Sodium                      | 0.001 g/l       | Resazurin Sodium                            | 0.001 g/l        | Resazurin sodium                                                | 0.001 g/l |
| Agar                                  | 0.75 g/l        | Agar (granulated)                           | 0.75 g/l (0.5-2) | Agar-Agar***                                                    | 0.75 g/l  |
| Water                                 | 1000 ml/l       | Water                                       | 1000 ml/l        | Water                                                           | n/a       |
| pH at 25 °C                           | 7.1 ± 0.2       | pH at 25 °C                                 | 7.1 ± 0.2        | pH at 25 °C                                                     | 7.1 ± 0.2 |

\* Enzymatic digest of casein is equivalent to tryptone.

\*\* Glucose is equivalent to Dextrose.

\*\*\* Agar-Agar is equivalent to other different terms of agar.

## Preparation

Dissolve 30 g in 1 l of purified water. Heat in boiling water and agitate frequently until completely dissolved. Dispense into tubes. Autoclave 15 min at 121 °C.

The prepared media are clear or almost clear and yellowish.

The medium shall be de-aerated before use.

## Experimental Procedure and Evaluation

Depends on the purpose for which the medium is used, e.g. follow directions given by EP, USP, JP or by EN ISO 7937.

Inoculate the culture medium with the sample material taking care that the sample reaches the bottom of the tubes.

In order to ensure anaerobiosis, the medium can then be overlaid with 1 cm of sterile liquid paraffin or agar solution.

Incubate the inoculated containers, e.g. acc. to EP, USP, JP, at 30-35 °C.

Anaerobes grow in the lower part of the culture.

**Note:** Fluid Thioglycollate Medium cannot be used if more than the upper third of the tube content has turned pink (resazurin indicator), and if this coloration does not disappear after de-aeration by boiling once.

## Storage

Store at +15 °C to +25°C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According EP, USP, JP, self-prepared medium can be stored between +2 °C to +25 °C in a sterile, airtight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in a free flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

## Quality Control

| Function     | Control strains                | ATCC® | Incubation               | Method of control | Expected results |
|--------------|--------------------------------|-------|--------------------------|-------------------|------------------|
| Productivity | <i>Clostridium perfringens</i> | 13124 | 18-24 h at 36-38 °C      | Qualitative       | Growth           |
|              | <i>Clostridium sporogenes</i>  | 11437 | up to 3 days at 30-35 °C |                   |                  |
|              | <i>Clostridium sporogenes</i>  | 19404 |                          |                   |                  |
|              | <i>Bacillus subtilis</i>       | 6633  |                          |                   |                  |
|              | <i>Staphylococcus aureus</i>   | 6538  |                          |                   |                  |
|              | <i>Bacteroides vulgatus</i>    | 8482  |                          |                   |                  |
|              | <i>Kocuria rhizophila</i>      | 9341  |                          |                   |                  |
|              | <i>Pseudomonas aeruginosa</i>  | 9027  |                          |                   |                  |

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133 and the harmonized method of EP, USP and JP.

## Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5<sup>th</sup> ed. American Public Health Association, Washington, D.C.

European Directorate for the Quality of Medicines and Healthcare. (2014): The European Pharmacopoeia. 8<sup>th</sup> Ed. Chapter 2.6.1 Biological Tests: Sterility. Strasbourg, France.

FDA-BAM (2013): Chapter No. 16: *Clostridium perfringens*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony-count technique. EN ISO 7937:2004.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Japanese Ministry of Health, Labour and Welfare. (2011): The Japanese Pharmacopoeia. 16<sup>th</sup> Ed. Chapter 4.06 Sterility Tests. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

United States Pharmacopeial Convention. (2014): The United States Pharmacopeia 38/National Formulation 33, Supp. 2. Chapter <71> Sterility Tests. Rockville, Md., USA.

## Ordering Information

| Product                                                         | Cat. No.     | Pack size |
|-----------------------------------------------------------------|--------------|-----------|
| GranuCult™ Fluid Thioglycollate Medium acc. EP, USP, JP and ISO | 1.08191.0500 | 500 g     |
| GranuCult™ Fluid Thioglycollate Medium acc. EP, USP, JP and ISO | 1.08191.5000 | 5 kg      |
| Agar-Agar purified                                              | 1.01614.1000 | 1 kg      |
| Paraffin viscous                                                | 1.07160.1000 | 1 l       |

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