

## Agar medium N (Cetrimide) Cetrimide agar media (VII)

356-4805

### DEFINITION

Medium used for the detection of *Pseudomonas aeruginosa* in pharmaceutical products.

### STANDARDS

• **European Pharmacopeia 6.0** - Biological methods - **2.6.13.**: Microbiological test of non-sterile products (Detection of specified micro-organisms)

• **USP 30/NF 25 US Pharmacopeia and National Formulary (2007)**: Microbial Limit Tests (61) - Microbiological Tests

• **NF ISO 22717 – Cosmetics – Microbiology** – Detection of *Pseudomonas aeruginosa*

### PRINCIPLE

The presence of magnesium chloride and potassium sulfate favor the production of pyocyanin by *Pseudomonas aeruginosa*. The presence of cetrimide renders the medium inhibitor.

### PRESENTATION

Dehydrated (without glycerol)  
500 g **code 356-4805**

### STORAGE

- +15-25°C, in carefully-sealed bottles in a cool, dry place and protected from light
- Expiration date and batch number are shown on the package.

### TYPICAL FORMULA

Pancreatic hydrolysate of gelatine	20 g
Dipotassium sulfate	10 g
Magnesium chloride	1.4 g
Cetrimide	300 mg
Agar	13.6 g
Glycerol	10 ml
Distilled water	1,000 ml

Final pH (25°C) = 7.2 ± 0.2

*NB: the formula has been adapted to attain the required performance criteria.*

### OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- **TSB**  
Ready to use  
100 ml x 10 bottles (code 355-4967)  
10 ml x 25 tubes (code 355-3454)
- Dehydrated  
500 g (code 356-4144)

### • Distilled water

### EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Hotplate
- Mixer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- 100 ml bottles with autoclave-proof stoppers
- Sterile Pasteur pipettes or inoculating loop
- Water-bath precise to ±1°C
- Thermostatically-controlled incubator or incubation room, precise to ±1°C
- Autoclave
- All usual laboratory equipment

### PREPARATION OF DEHYDRATED MEDIUM

#### Always shake before use.

Dissolve 45.3 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix until the suspension is homogeneous. Add 10 ml of glycerol. Bring gently to boiling point until completely dissolved. Pour into tubes or bottles. Sterilize in autoclave at 121°C (± 1°C) for 15 minutes.

**Reconstitution ratio: 45.3 g/l**  
**500 g of powder makes 11 liters of medium.**

### PROTOCOL

#### Inoculation and incubation

Inoculate 100 ml of TSA agar (see corresponding Technical Sheet) with 10 ml of sample or the quantity corresponding to 1 g or 1 ml of product. Homogenize and incubate at 36°C (± 1°C) for 18 to 48 hours. Carry out subcultures on Cetrimide agar medium and incubate at 36°C (± 1°C) for 18 to 72 hours.

*NB: For examination of transdermic devices, filter 50 ml of Preparation A (as described in general method 2.6.12.) on a sterile membrane and transfer the membrane to 100 ml of TSA medium. Incubate at 36°C ( $\pm$  1°C) for 18 to 48 hours, then spread on Cetrimide agar medium. (Pharmacopée Européenne - Addendum 2001 - 2.6.13.).*

#### **READING AND INTERPRETATION**

The product satisfies the test if no microbial growth is observed. If colonies with Gram-negative rods appear, inoculate the TSB liquid medium with some of the morphologically different, isolated colonies and incubate at 42°C ( $\pm$  1°C) for 18 to 24 hours. The product satisfies the test if there is no growth at 42°C ( $\pm$  1°C) (Pharmacopée Européenne – Addendum 2001 - 2.6.13.).

#### **QUALITY CONTROL**

In view of the current harmonization of pharmacopeias, we recommend that you refer to the certificates of analysis for procedures relating to the quality control (performance and selectivity) of media produced by Bio-Rad.

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

#### **KEY WORDS**

Cetrimide/Pharmaceutical products/  
*Pseudomonas aeruginosa*/Pyocyanin/  
Glycerol/Medium