**Intended Use:**
Recommended for the routine qualitative detection of residues from antibiotics and other chemotherapeutic agents in animal-derived food.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM peptone #</td>
<td>7.800</td>
</tr>
<tr>
<td>Casitose ##</td>
<td>7.800</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.800</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>1.000</td>
</tr>
<tr>
<td>Starch</td>
<td>4.000</td>
</tr>
<tr>
<td>Gelatin</td>
<td>4.000</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>0.016</td>
</tr>
<tr>
<td>Agar</td>
<td>10.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 40.41 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**
Kundrat Agar is used for detection of antimicrobial residues in animal feed preparations. The test is carried out using a spore suspension of *Bacillus stearothermophilus* as test microorganisms. It is also used for detection of antimicrobial residues in meat and organ samples; used together with spore suspensions of *Bacillus subtilis* (BGA) as test organism. Presence of chemotherapeutic agents is indicated by the formation of inhibition halos or zones around the disc with the sample (3).

Cleaning agents, disinfectants and preservatives are not covered by this test. When performing the rapid test, pre-incubating the inoculated plates enhances growth of the test organism; the inhibition zones then appear more rapidly after application of the samples.

The test is performed in the form of an agar diffusion test. Any inhibitors present produce inhibition zones devoid of bacterial growth surrounding the applied samples. With further incubation, the test organism ferments glucose present in the medium to form acid, that causes bromocresol purple to change its colour to yellow. Only the inhibition zone still retains the original violet colour of the indicators.

Test Procedure: After autoclaving the medium, cool to 50-60°C. To each 200 ml of the medium add $10^5$ spore suspension of *B. stearothermophilus*, mix, pour in plates.
Filter paper discs with a diameter of 6 mm are soaked with the liquid specimen or placed on organ (kidney, liver) or muscle sections. The discs are then slightly pressed onto the surface of the culture medium (up to 6 discs per plate) (4).
Two methods are recommended for performing the test:
1) 45 minutes incubation, rapid test;
After placing the discs on the preincubated plates, incubate them for further 45 minutes at 65°C without prediffusion.
2) 3 hour incubation:
The plates are not preincubated. After the filter paper discs have been applied to the plates, they should be incubated for 3 hours at 65°C without pre-diffusion.
In the case of rapid test, formation of inhibition zones can be seen after 15-25 minutes incubation in the medium, which is otherwise turbid as a result of spore growth. After the 45 minutes incubation, the inhibition zones become even more distinct due to the fact that the culture medium changes colour. Formation of inhibition zones is to be regarded as a positive result. In the case of the 3 hours incubation, only those inhibition zones with a diameter of more than 10 mm can be considered positive. If a distinct colour change has not occurred after 45 minutes or 3 hours, incubation can be prolonged.

**Type of specimen**
Food samples- Meat and Meat products

**Specimen Collection and Handling**
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions :**
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations :**
1. Some organism may show poor growth due to nutritional variation.

**Performance and Evaluation**
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

**Quality Control**

**Appearance**
Cream to yellow coloured with green tinge, homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.0% Agar gel.

**Colour and Clarity of prepared medium**
Light purple coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.04% w/v aqueous solution at 25°C. pH : 6.8±0.2

**pH**
6.60-7.00

**Cultural Response**
Cultural characteristics observed after an incubation at 65°C for 18-24 hours.

**Organism**
**Growth**

*Bacillus stearothermophilus* good-luxuriant
*ATCC 7953*

**Storage and Shelf Life**
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

**Disposal**
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).
Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition