

## Inhibitor Test Agar (pH 7.2)

### DM298

#### Intended Use

A standardised medium for the detection of antimicrobial inhibitors in meat and organ samples by the 3-plate test method.

#### Contents

See pack label.

#### Formulation\*

Material:	Concentration in medium:
Peptone mixture	7.0 g/litre
Sodium chloride	5.0 g/litre
Tri-sodium phosphate	0.8 g/litre
Agar	13.0 g/litre
Final pH: 7.2 ± 0.2	

#### Storage and shelf life

All dehydrated culture media containers should be kept tightly closed and stored in a dry place at 10 to 25°C until the expiry date shown on the pack label.

#### Precautions

For *in vitro* diagnostic use only. Observe approved hazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Refer to Product Safety Data sheet (available on request or via MAST® website).

#### Materials required but not provided

Standard microbiological supplies and equipment such as loops, MAST® selective supplements, swabs, applicator sticks, incinerators and incubators, etc., as well as serological and biochemical reagents and additives such as blood.

#### Procedure

1. Refer to pack label for quantities and volumes required. Prepare MAST® Inhibitor Test Agar (pH 7.2) (DM298D) by suspending the powder in distilled or deionised water. For sachet packs, dissolve the entire contents of the sachet in the volume shown on the label.
2. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
3. Cool to 50 to 55°C and hold at this temperature in a water bath.
4. Add 1ml/litre of a *Bacillus subtilis* (BGA) (DSM No 618) spore suspension containing approximately 1x10<sup>7</sup> CFU/ml (final concentration in the medium of 1x10<sup>4</sup> CFU/ml)

5. Add 1ml/litre of a trimethoprim solution containing 50mg/litre (final concentration in the medium of 50µg/litre). To prepare a trimethoprim stock solution, dissolve 10 mg of Trimethoprim in 10ml of ethanol at 50°C. Add 190ml of sterile deionised or distilled water to make a ready-to-use solution of 50mg/litre. Stored at 2 to 8°C this solution can be kept for 14 days. Alternatively, one MAST® Trimethoprim 0.05mg ADATAB® (TAB/TM0.05) can be added to 1 litre of prepared medium. If smaller volumes of medium are to be prepared the MAST ADATAB® can be dissolved in a small quantity of ethanol (0.2ml to 1.0ml), aliquotted for a single use and stored at -20°C until required.
6. Pour culture plates 2mm thick (10 to 15ml per 9cm Petri dish) and allow to set.
7. Prepared culture plates should be used immediately.
8. Prepare meat samples by cutting 2mm thick slices from a sterile cylindrical core of tissue 8mm in diameter. A total of 6 discs are required for the complete 3-plate test (Inhibitor Test Agars pH 6.0, pH 7.2 and pH 8.0 - DM276D, DM298D and DM308D respectively).
9. Using sterile forceps, place two of the discs in diametrically opposite positions on each test plate.
10. Using sterile forceps, place appropriate control discs (For MAST® Inhibitor Test Agar (pH 7.2) use Sulphadimidine 0.5µg) on each of the three test plates.
11. Incubate plates of MAST® Inhibitor Test Agar (pH 7.2) at 30°C for 18 to 24 hours.

#### Interpretation of results

After incubation measure and record any zones of inhibition formed. A positive result is indicated by an annular zone of complete inhibition of growth, not less than 2mm wide, around both meat discs from a sample. An annular zone of inhibition of less than 2mm but greater than 1mm is a borderline result.

#### Quality control

Check for signs of deterioration. Quality control must be performed with appropriate control discs to demonstrate expected performance. Do not use the product if the result with a control disc is incorrect. The table below describes a performance control susceptibility test disc for routine use, which can be easily obtained as part of the MASTDISCS™ range by the end user:-

Antimicrobial agent and content of disc	Zone of inhibition (radius)
Sulphadimidine 0.5µg (SD0.5)	>6mm

#### References

Bibliography available on request.