



## PRODUCT SPECIFICATION SHEET

### Antimicrobial Inhibitor Test Agar pH 7.2 (DM1328)

#### Intended Use

Antimicrobial Inhibitor Test Agar pH 7.2 (DM1328) is recommended for antimicrobial residual analysis in meat and organ samples, using *Bacillus subtilis* (ATCC 6633) as test organisms.

#### Product Summary and Explanation

In order to sanitize muscle foods, treatments with antimicrobial compounds such as chlorine and organic acids are used in addition to washing. Diverse concentrations and degree of effectiveness of the concentrations of these antimicrobial compounds have been reported. Antimicrobial Inhibitor Test Agar pH 7.2 is recommended for residual analysis of antimicrobial components in meat and organ samples, by agar diffusion procedure and EEC Four-Plate-Test using *Bacillus subtilis* (ATCC 6633) as test organism.

#### Principles of the Procedure

Antimicrobial Inhibitor Test Agar pH 7.2 contains tryptone, meat extract which provides essential nitrogenous and carbonaceous compounds and other needed nutrients for the growth of organisms. Sodium chloride helps to maintain osmotic balance in the medium.

#### Formula / Liter

Ingredients	Gms / Liter
Peptone	7.00
Trisodium phosphate, 12H <sub>2</sub> O	0.80
Sodium chloride	5.00
Agar	13.00
Final pH: 7.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

#### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

#### Directions

1. Suspend 25.34 grams of the medium in one litre of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C.
5. Mix 1 ml of *Bacillus subtilis* spore suspension per liter of sterile and cooled (45-50°C) medium. Add 50µg of trimethoprim per litre of medium.
6. Mix thoroughly and pour into sterile Petri plates.

#### Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 2.53% Solution	pH : 7.20 at 25°C
Gel Strength	Firm, comparable with 1.3% Agar gel



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**Expected Cultural Response:** Cultural response and zone of inhibition observed after an incubation at 30°C using *B.subtilis* 18-24 hours.

Sr. No.	Organisms	Results to be achieved				
		Growth	Inhibition zones with Penicillin (10 IU)	Inhibition zones with Streptomycin (10mcg)	Inhibition zones with Streptomycin (0.5mcg)	Inhibition zones with Sulphadiazine (0.5mcg)
1.	<i>Bacillus subtilis</i> ATCC 6633	good-luxuriant	25-35	25-30	15-20	12-20

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

#### Four-Plate-Test:

##### a) Agar Diffusion procedure:

1. Place small slices of the meat sample on the seeded Test agar plates and incubate the plates.
2. If any antimicrobial components are present in the sample it diffuses into the medium and inhibits the growth of test organisms causing growth free inhibition zone.
3. This test is repeated with all the three Antimicrobial Inhibitor Test Agar with three different pH ie. DM1024 (pH 6.0), DM1328 (pH 7.2) and DM1025 (pH 8.0).
4. Different antibiotic to be analysed such as penicillin, streptomycin and sulphonamide have different pH range for optimal activity for example penicillin G is active optimally at pH 6.0 and streptomycin at pH 8.0 and sulphonamide at pH 7.2.

##### b) For EEC Four-Plate-Test method:

1. Molten and cooled antimicrobial inhibitor test agar is seeded with test organisms, *Bacillus subtilis* spores suspension,<sup>(1)</sup> mix well and dispense in petri plates.
2. In one half of the seeded plate, aseptically place two small piece (2-8mm) of meat or organ sample at proper distance and in second half of the plate, place test discs with standard antibiotic to be analyzed, as control. Use disc with 0.01 IU of penicillin-G sodium salt as standard.
3. Incubate the plates for 18-24 hours at 30°C for *Bacillus subtilis*. After incubation measure the zone of inhibition. The inhibition zone between tissue section edge and growth limit of test organism is measured.
4. The zone of at least 2 mm is regarded as positive and less than 2mm (12mm) is considered doubtful. The standard disc should display minimum 6 mm zone of inhibition.

Refer appropriate references for standard test procedures.

### Results

Refer appropriate references and test procedures for interpretation of results.

### Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.



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### Packaging

Product Name : Antimicrobial Inhibitor Test Agar pH 7.2

Product Code : DM1328

Available Pack sizes : 500gm

### References

1. Ferrini, A. M.; Mannoni, V., Aurdi P. Combined plate microbial assay (CPMA). Food additives and Contaminants, 23(1):16-24. 2006.

### Further Information

For further information please contact your local MICROMASTER Representative.



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