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DNase Agar – DM132

Introduction

MAST® DNase Agar is a biochemical test medium that provides information on the metabolic action of an organism to aid differentiation and identification of microbiological culture isolates. DNase Agar gives typical reactions when a separate developing reagent , 1M hydrochloric acid, is added following incubation. MAST® culture media is supplied in a dehydrated powder form, allowing the end-user to prepare a suitable medium for bacterial & fungal culture. It is suitable to be prepared in a variety of receptacles and at volumes that conform to the end-users desired purpose. The culture of bacterial and fungal species are essential for routine clinical laboratory purposes.

FOR IN VITRO USE ONLY
NOT FOR USE IN DIAGNOSIS OF HUMAN DISEASE

Intended Purpose

DNase Agar dehydrated culture medium powder is intended for use to produce a medium to aid differentiation of members of *Staphylococcus aureus* from other staphylococci. This medium is used to determine the ability of an organism to produce deoxyribonuclease (DNase), an enzyme capable of degrading deoxyribonucleic acid (DNA).

When prepared in accordance with the instructions for use, it produces a semi-solid medium capable of supporting the growth of staphylococci. The metabolic action of an organism able to produce extracellular DNase results in hydrolysis of the DNA contained in the medium Oligonucleotides liberated by hydrolysis of DNA are soluble in acid and when combined with a separate developing reagent, 1M hydrochloric acid, a clear zone is formed around the inoculum which is interpreted as a positive result. Precipitation of intact DNA by hydrochloric acid, in a negative reaction, results in the medium becoming cloudy. This medium can also be used in conjunction with additional identification products to produce a phenotypic biochemical profile of the bacterial isolate.

DNase Agar is intended to be used in conjunction with other phenotypic tests to aid epidemiological typing of previously isolated and identified pure cultures of *Staphylococcus* spp. derived from, animal, food or environmental samples. It is intended to be used by professional, trained laboratory users for *in vitro* use and is not intended for use in the diagnosis of disease or other conditions in humans or as the basis of treatment or case management decisions.

Principle of the test

Culture media remains the gold standard for the growth and isolation of viable bacterial and fungal cells. Plates are inoculated with the target organism or specimen by surface plating. Plates should be incubated under the appropriate atmospheric conditions and temperature for the target organism(s). Interpretation of cultures following incubation requires significant skill on behalf of the operator in the determination of additional procedures required. This determination is reliant upon growth characterisitics of the microorganism including such as morphology and observing changes in the media surrounding the colonies.

These methods should be used in conjuction with other *in vitro* devices in the aid of diagnosis.

Once prepared a single culture media plate is only for single use and cannot be re-used.

Components

MAST® culture media is supplied in a dehydrated form for reconstitution by the end-user. The formulation of the product is described in Table 1.

Table 1. Formulation of DM132*

Material	Concentration in medium
Selected peptone mixture	20.0 g/L
Sodium chloride	5.0 g/L
Deoxyribonucleic acid	5.0 g/L
Agar	14.0 g/L

*Formulation may change to meet performance criteria.

The formulation is illustrative of the DM132 product range. The product is manufactured within an ISO:9001 and ISO:13485 environment. Inter-batch variation is expected to be minimal with no direct impact on the product

Stability and storage

The expiry date applies to unopened containers of MAST® dehydrated culture media when stored in the primary container and in accordance with the manufacturer's instructions. The expiry date and batch number are indicated on each pack label.

- Store packs in a dry environment.
- Store packs at room temperature (10°C to 25°C).
- Avoid sources of moisture such as autoclaves, CO2 incubators and water-baths.
- Limit the time a pack remains open whilst in use.
- This product is hygroscopic, avoid prolonged exposure to ambient moisture.
- For opened packs of dehydrated culture media ensure lid is firmly closed after every use.

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- Before use ensure the appearance of the media conforms to the expected colour and texture i.e. free flowing, no excessive lumps. Media that is discoloured or lumpy should be further examined for performance against the recommended QC organism panel.

Warnings and precautions

1. DNase Agar is for *in vitro* use only, and must be used by trained professional laboratory staff.
2. All microbiological cultures and equipment used to transfer and manipulate them should be treated as infectious. Autoclave sterilise all biohazard waste before disposal in accordance with local regulations.
3. On receipt, store MAST® dehydrated culture media at the recommended storage temperature and conditions stated on the pack.
4. Do not store near sources of moisture or within high humidity environments.
5. Do not use if media powder is discoloured and/or lumpy, examine against recommended QC organism panel before continuing use. Discolouration could be a sign of degradation and must be examined further.
6. When handling the device ensure that local and regulatory health and safety advice is followed.
7. When handling the sterilised solution, beware of the temperature, use thermal resistant gloves where appropriate.
8. When preparing culture media after sterilisation, ensure that this is performed in an aseptic manner.
9. Observe recommended safe laboratory practices when preparing and handling 1M hydrochloric acid reagent.
10. DNase Agar is not intended for use in the diagnosis of disease or other conditions in humans or as the basis of treatment or case management decisions.

MAST® dehydrated culture media are supplied in a sealed primary container, which helps to prevent moisture ingress from the environment. The nature and frequency of use of the device is conducive to an end-user re-entering the container. When the product is not in use, the primary container should remain sealed.

Materials Provided

Mast® dehydrated culture media is supplied in a powder form contained within a re-usable primary container for end-user reconstitution.

Materials required but not provided

Standard microbiological supplies and equipment such as, petri dishes, bottles, tubes, laminar flow cabinet, water bath, autoclave, balance, weigh boats, spatulas, thermometer, developing reagent: 1M hydrochloric acid, deionised water, or suitable control strains of microorganisms.

Procedure

1. Refer to pack label for quantities and volumes required. Prepare MAST® *DNase* Agar (DM132) by suspending the powder in distilled or deionised water.
2. Sterilise by autoclaving at 121°C (15 p.s.i.) for 15 minutes.
3. Cool the medium to 50 to 55°C and hold at this temperature in a water bath until ready to pour culture media plates
4. Mix well and pour culture to a volume of 15 to 20ml per 9cm Petri dish and allow to set.
5. Prepared culture plates may be used immediately or stored in plastic bags at 2 to 8°C for up to one week.

Refer to local Health and Safety handling procedures for infectious waste disposal guidelines.

Technical guidance

Observe the powder before use. If the powder is discoloured or lumpy, this could be a sign of degradation and must be further examined.

Interpretation of results

After incubation and addition of 1M hydrochloric acid record zones of clearing around each growth spot or streak of growth when observed against a dark background. A clearly defined zone of clearing indicates DNA has been broken down into nucleotide fractions which are not precipitated by acid. Record these organisms as DNase positive. DNase negative colonies show no or only narrow zones of clearing.

Limitations of use

1. 1M HCl is bactericida. Once the hydrochloric acid reagent has been applied, the test cannot be continued by reincubation or cultures recovered from the test plate.
2. Some strain of Methicillin Resistant *Staphylococcus aureus* (MRSA) produce a negative DNase reaction
3. Strains of *Staphylococcus intermedius* and certain strains of *Staphylococcus hyicus* (both coagulase positive) are DNase positive
4. Some strains of coagulase negative staphylococci such as *Staphylococcus capitis*. may give weak positive reactions

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5. MAST® DNase agar is intended to be used in conjunction with other phenotypic tests to aid differentiation and epidemiological typing of previously isolated and identified pure cultures of *Staphylococcus* spp.

MAST® media are not intended to be used as the sole, and primary isolation medium in instances where a failure to detect a pathogenic infection would result in death, serious illness or possible transmission of infectious disease.

Quality control *(Check strains vs SMI vs QC)*

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate expected performance. Do not use the product if the result with the control organism is incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Table 2. Suggested organisms for QC

Test Organisms	
Staphylococcus aureus ATCC® 9144	Positive
Staphylococcus aureus ATCC® 25923	Positive
Staphylococcus epidermidis ATCC® 14990	Negative

References

Bibliography available on request.

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