

DNA/Agar

355-5552
356-4404

DEFINITION

Desoxyribonucleic acid (DNA) agar is a solid medium permitting detection of desoxyribonuclease of bacteria, in particular that of staphylococci.

PRINCIPLE

The presence of DNA in the medium enables detection of desoxyribonuclease. The microorganisms are streak inoculated over the surface of the agar and incubated.

After incubation, the surface of the agar is flooded with hydrochloric acid or with toluidine blue solution.

In the presence of HCl, polymerized DNA precipitates and the medium becomes opaque. If the bacteria produce sufficient desoxyribonuclease, a clear halo (due to DNA hydrolysis) appears around the colonies.

PRESENTATION

• Ready-to-use

100 ml x 6 bottles

code 355-5552

• Dehydrated

500 g

code 356-4404

STORAGE

- Ready-to-use: + 2°C to 8°C.
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	20 g
DNA	2 g
Sodium chloride	5 g
Agar	12 g
Distilled water	1,000 ml
Final pH (25°C) = 7.3 ± 0.2	

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- **Chapman Mannitol /Agar**
 - 55 mm x 10 plates (code 356-3926)
 - 500 g (code 356-4824)
- **Baird Parker /Agar**
 - 500 g (code 356-4814)
 - 90 mm x 20 plates (code 356-3991)

See corresponding Technical Sheet(s)

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Hotplate
- Mixer-homogenizer
- 125 ml bottles with autoclave-proof stoppers
- Sterile pipettes (0.1 ml, 1 ml, etc)
- Sterile Petri dishes (Ø= 90 mm)
- Sterile Pasteur pipettes or inoculating loops
- Sterile spreaders
- 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 well before use.

Dissolve 39 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to boiling point until completely dissolved. Dispense, then sterilize in autoclave at 121°C (± 1°C) for 15 minutes.

Reconstitution ratio: 39 g/l
500 g of powder makes 12.8 liters of medium.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

• Inoculation and incubation

Collect the suspect colonies on Chapman medium (codes 356-3926, 356-4824) or Baird Parker medium (for *S. aureus*) (codes, 356-4814, 356-3991) and inoculate on the surface of the dish in a single streak 2 cm long or in groups of 1 cm diameter. Incubate at 37°C (± 1°C) for 24 hours.

N.B.: 4 or 5 colonies can be inoculated onto the same dish.

READING AND INTERPRETATION

After incubation, flood the dishes with standard hydrochloric acid solution or with 0.1% solution of toluidine blue.

In the next 5 minutes, various phenomena can be observed:

- **Detection using hydrochloric acid:**

- Clear zone around the streak, with the rest of the plate remaining opaque: the strain is desoxyribonuclease-positive.
- No clear zone around the streak: the strain is desoxyribonuclease-negative.

- **Detection using toluidine blue:**

- Pink zone around the streak, with the rest of the plate remaining blue: the strain is desoxyribonuclease-positive.
- No pink zone around the streak: the strain is desoxyribonuclease-negative.

N.B.: Strains of Staphylococci which have coagulase generally also possess desoxyribonuclease. This medium is also suitable for confirming diagnosis of Serratia marcescens or S. liquefaciens: DNase + Enterobacteria.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains.

STRAINS	Results after 24h culture at 37°C	
	Growth	DNase
<i>Staphylococcus aureus</i> ATCC 25923	+	+
<i>Staphylococcus epidermidis</i> ATCC 14990	+	-
<i>Serratia marcescens</i> ATCC 8100	+	+

QUALITY CONTROL OF MANUFACTURER

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

AND / *Staphylococcus aureus* / *Staphylococci* / Detection / Deoxyribonuclease / HCl / Toluidine blue / Medium.

BIBLIOGRAPHY

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- **BLAIR E.B, EMERSON J.J and TULL A.H.** (1967): Am. J. Clin. Path. 47 : 30-39.