# AZIDE MALTOSE AGAR (KF)

**Selective medium for the isolation and enumeration of enterococci**

**Typical formula (g/l)**

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| Peptocomplex | 10 |
| Yeast Extract | 10 |
| Sodium Chloride | 5 |
| Na Glycerophosphate | 10 |
| Maltose | 20 |
| Lactose | 1 |
| Agar | 15 |
| Sodium Azide | 400 mg |
| Bromocresol Purple | 15 mg |

**Directions**

Suspend 71.4 g in 1000 ml of cold distilled water, heat to boiling, and boil for five minutes, cool in a water bath to 50°C and aseptically add 10ml of TTC 1% Solution (code 42111801). Mix well and pour into sterile 55mm Petri dishes for water analysis, or into flasks for poured plated technique. The medium without TTC can be autoclaved at 121°C for 10 minutes.
Final pH 7.2 ± 0.2

**Description**

Azide Maltose Agar, prepared according to the formula of Kenner, Clark and Kabler, is a selective medium used for the isolation and enumeration of enterococci in faeces, milk , water and other materials by plate pouring or membrane filtration techniques. Azide Maltose Agar KF complies with the recommendations given by APHA for the examination of water and foodstuffs. Enterocci metabolize maltose and lactose with the production of acid and reduce TTC to give a red formazan and thus appear as red colonies with yellow zones.

**Technique**

**Membrane Filtration Method**

1. Distribute 4-5ml of medium into 55mm dishes
2. Filter samples through a sterile membrane to give 20 to 200 colonies on the membrane surface. Use volume of 100, 10, 1, 0,1 or 0,01, depending on the degree of pollution.
3. Transfer the membrane to the agar, invert the plates and incubate at 35°C for 48 hours.
4. Count all red or pink colonies eventually with the aid of a low power (10 to 15 magnifications) microscope.
5. Calculate the number of enterococci and report as faecal streptococci per 100 ml.

**Plate Count Method**

1. Prepare dilutions of the sample to give a count of 30-300 colonies.
2. Transfer 1ml of sample dilutions in duplicate into sterile Petri dishes
3. Pour 20ml of liquefied and cooled to 45-47°C medium to each plate.
4. Mix the medium well with the sample and solidify the agar as quickly as possible.
5. Incubate the inverted plates at 35°C for 48 hours.
6. Count all red or pink colonies eventually with the aid of a low power (10 to 15 magnifications) microscope.
7. Calculate the number of enterococci and report as faecal streptococci per 100ml.

For confirmation of colonies a serological or biochemical test is recommended. For biochemical confirmation, transfer 5-10 typical colonies to Brain Heart Infusion Broth and incubate for 18-24 hours at 35°C. Use the broth culture for a Gram stain and a catalase test, a subculture into Brain Heart Infusion Broth (incubate at 45°C), and into the same medium with bile salts.
The diagnosis of faecal streptococci is given by a negative catalase reaction, growth in BHI incubated at 45°C, and in BHI with bile salts after 72 hours at 35°C.

**Precautions**
Azide Maltose Agar KF is not specific for the identification of enterococci and a serological confirmation must be carried out. The pH of the medium should not fall below 7.0, as it may become inhibitory towards enterococci.

**User quality assurance** (37°C-24 h)
Productivity control
*E.faecalis* ATCC 19433: good growth, red colonies
Selectivity control
*E.coli* ATCC 25922: inhibited

**Storage**
Dehydrated medium: 10-30°C
User prepared plates and flasks: 30 days at 2-8°C

**References**
• APHA (1999). Standard Methods for Examinations of Water and Waste-water, 20th edition
• APHA (1992). Compendium of Methods for Microbiological Examination of Foods, 3rd ed.
• Hartman, P.A., Reinbold G.W., & Saraswat, D.S. (1966) Adv. App, Micr., **8**, 253
• Kenner, B.A., Clark, H.F. & Kabler, P.W. (1961). App. Microbiol., **9**,15.

**Packaging**

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| **4011072** | **Azide Maltose Agar KF,** | **500 g (7 l)** |
| **4911072** | **Azide Maltose Agar KF,** | **30 ready to use plates Ø 55 mm** |