

TECHNICAL DATA SHEET

KF STREPTOCOCCUS AGAR (BASE)

ENUMERATION OF *ENTEROCOCCI*

1 INTENDED USE

KF (Kenner Fecal) Streptococcus Agar is a selective medium used for the isolation and enumeration of enterococci in food products by using classic enumeration techniques on Petri plates.

It is also recommended in the standard for the composition of acidifying lactic starters in dairy products (ISO 27205), for the detection of enterococci as contaminants.

2 HISTORY

KF Agar used by Kenner *et al.* in 1960 for the enumeration of fecal streptococci in water samples was found to have an excellent recovery power in comparison to other media used at that time. The authors observed that the medium led to an excellent characterization of enteric cocci as well as of microorganisms with similar biochemical and antigenic properties : *Streptococcus bovis* and *Streptococcus equinus*.

3 PRINCIPLES

The high nutritive capacity of the medium is due to the presence of a high proportion of polypeptone, yeast extract and carbohydrates.

Sodium chloride maintains the osmotic equilibrium.

Lactose and maltose are energy sources for microorganisms that can use them.

The acidification of the medium is shown by bromocresol changing from purple to yellow.

Sodium azide inhibits the growth of contaminating Gram-negative bacteria.

TTC added just before use is an indicator of bacterial growth. It is reduced to an insoluble formazan inside cells. This reaction is visualized by the appearance of red to maroon colonies.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

| | |
|---|---------|
| - Polypeptone | 10,0 g |
| - Yeast extract | 10,0 g |
| - Maltose..... | 20,0 g |
| - Lactose..... | 1,0 g |
| - Sodium chloride | 5,0 g |
| - Sodium glycerophosphate..... | 10,0 g |
| - Sodium azide | 0,4 g |
| - Bromocresol purple | 15,0 mg |
| - 2, 3, 5 triphenyltetrazolium chloride | 0.1 g |
| - Bacteriological agar..... | 12,0 g |

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 68,4 g of dehydrated base media BK132

| | |
|---------------------------------|---------|
| - Polypeptone | 10,0 g |
| - Yeast extract | 10,0 g |
| - Maltose | 20,0 g |
| - Lactose | 1,0 g |
| - Sodium chloride | 5,0 g |
| - Sodium glycerophosphate | 10,0 g |
| - Sodium azide | 0,4 g |
| - Bromocresol purple | 15,0 mg |
| - Bacteriological agar | 12,0 g |

For one vial of supplement BS027

- Triphenyltetrazolium.....50 mg
(chloride)

5 PREPARATION

- Dissolve 68,4 g of dehydrated base media (BK132) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring until complete dissolution.
- Continue boiling for 5 minutes without exceeding this period of heating.
- Do not autoclave.
- Cool and maintain in molten state at 44-47 °C
- Rehydrate the TTC supplement (BS027) with 5 mL sterile distilled or demineralized water.
- Mix or vortex the supplement vial to insure complete dissolution, while avoiding foam formation.
- Add 1 mL of reconstituted supplement to 100 mL of base media held at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.

✓ **Reconstitution :**
68,4 g/L

✓ **Sterilization :**
Do not autoclave

✓ **Rehydration supplement :**
5 mL sterile distilled water
✓ **Add to base :**
1 mL / 100 mL

6 INSTRUCTIONS FOR USE

- Transfer 0.1 mL of the sample to be tested and its serial dilutions to the surface of the agar.
- Spread over the surface with a sterile triangle or "hockey stick".
- Incubate at 43 ± 1 °C for 48 hours.

✓ **Inoculation**
0,1 mL on surface

✓ **Incubation**
48 h at 43 °C

NOTE :

The media can be inoculated using the pour plate technique as well, with 1 mL of inoculum per plate.

7 RESULTS

Colonies that are red, maroon or pink, surrounded by a yellow halo, are considered characteristic.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : whitish powder, free-flowing and homogeneous.

Freeze-dried supplement : white pellet, after reconstitution giving a transparent, limpid solution.

Prepared (complete) media : violet-blue agar.

Typical culture response after 48 hours of incubation at 43 °C :

| Microorganisms | | Growth (Productivity Ratio : P_R) |
|------------------------------|------------|---|
| <i>Enterococcus faecalis</i> | WDCM 00087 | $P_R \geq 70\%$ |
| <i>Enterococcus faecium</i> | WDCM 00010 | $P_R \geq 70\%$ |
| <i>Escherichia coli</i> | WDCM 00013 | Inhibited, score 0 |
| <i>Staphylococcus aureus</i> | WDCM 00034 | Inhibited, score 0 |

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.

TTC 50 mg Supplement: 2-8 °C.

The expiration dates are indicated on the labels.

Prepared, complete media in plates (*) : 8 days at 2-8 °C, shielded from light.

Rehydrated supplement (*) : 30 days at 2-8 °C shielded from light.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK132HA

TTC 50 mg Supplement:

10 vials BS02708

11 BIBLIOGRAPHY

Kenner, B.A., Clark, H.F., and Kabler, P.W. 1961. Fecal Streptococci. I. Cultivation and Enumeration of Streptococci in Surface Waters. Appl. Microb., 9: 15-20.

MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Maintenance of Medical Bacteria. Vol 1. Williams and Wilkins, Baltimore, 395-398.

ISO 27205. Février 2010. Produits laitiers. Ferments acidifiants. Norme de composition.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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Origin of revision : General update.

ANNEX 1 : PHOTO SUPPORT

KF AGAR

Detection and enumeration of enterococci.

Results :

Growth obtained after 48 hours of incubation at 43 °C.

