# CM 20252 – BILE ESCULIN AGAR W/ KANAMYCIN

## INTENDED USE

For selective isolation and presumptive identification of Bacteroides fragilis from mixed flora.

## PRODUCT SUMMARY AND EXPLANATION

GroupDStreptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci. The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld. Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate. The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix. Bile Esculin Agar was originally formulated by Swan for the isolation and identification of Group D Streptococci from food. Facklam and Moody further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non-Group D Streptococci. Bile Esculin Agar was also shown to aid differentiation of Enterobacteriaceae, Klebsiella, Enterobacter, Serratia from other Enterobacteriaceae genera on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci.

Bile Esculin Agar with Kanamycin is recommended for the selective isolation and presumptive identification of Bacteroides fragilis group of bacteria from mixed flora. This medium is a modification of the original formulation of Swan. In this medium kanamycin is added to an enriched Bile Esculin Agar, enriched with hemin and vitamin K1. Hemin and vitamin K1 enriches and enhances the growth of Bacteroides species. Kanamycin selectively promotes the growth of Bacteroides fragilis while inhibiting the growth of facultative anaerobic and aerobic gram-negative bacilli. Anaerobes that are incapable of hydrolyzing esculin do not form brown or black pigmented colonies on this medium. The plates should be reduced by keeping in anaerobic jar for 18-24 hours, just before incubation.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| Gelatin peptone | 5.000 |
| Beef extract | 3.000 |
| Oxgall | 20.000 |
| Ferric citrate | 0.500 |
| Esculin | 1.000 |
| Kanamycin | 0.100 |
| Hemin | 0.010 |
| Vitamin K1 | 0.010 |
| Agar | 15.000 |

## PRINCIPLE

Gelatinpeptone and meat peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and kanamycin inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. Viridans Streptococci sometimes exhibit a weak positive reaction.

## INSTRUCTION FOR USE

Dissolve 44.6gramsin1000 ml purified / distilled water.
Heat to boiling to dissolve the medium completely.

- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45 - 50°C.
- Mix well and pour into sterile Petri plates. DO NOT OVERHEAT.

## QUALITY CONTROL SPECIFICATIONS

| | |
|---|---|
| Appearance of Powder | : Light yellow to brownish yellow homogeneous free flowing powder. |
| Appearance of prepared medium | : Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates. |
| pH (at 25°C) | : 7.1±0.2 |

## INTERPRETATION

Culturalcharacteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Esculin hydrolysis | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|---|---|
| Bacteroides fragilis | 25285 | 50-100 | Luxuriant | >=70% | Positive reaction, blackening of medium around the colony | 35-37°C | 24-48 Hours |
| Escherichia coli | 25922 | 50-100 | None-poor | 0-10% | Negative reaction | 35-37°C | 24-48 Hours |
| Fusobacterium necrophorum | 25286 | 50-100 | None-poor | 0-10% | Negative reaction | 35-37°C | 24-48 Hours |

## PACKAGING:

Inpacksizeof100 gm and 500 gm bottles.

## STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

Afteruse,prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

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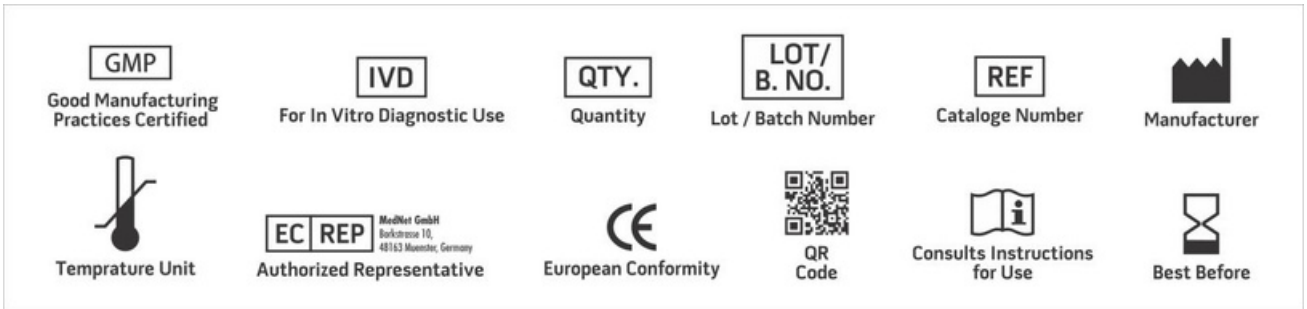


NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For LabUse Only