TECHNICAL DATA SHEET

COMPASS® *ECC* AGAR

ENUMERATION OF *ESCHERICHIA COLI* AND OTHER COLIFORMS

1 INTENDED USE

The chromogenic media COMPASS® *Ecc* Agar is a selective agar for the simultaneous and specific enumeration without confirmation of *Escherichia coli* and of other coliform bacteria in human and animal food.

2 HISTORY

The classification of coliforms is traditionally founded on their capacity to ferment lactose with a corresponding production of acid. The fermentation of lactose results from the successive cascade effect of two enzymes : first a permease responsible for the penetration of the sugar into the bacteria, and then a β-galactosidase which cuts the glucose to galactose, thereby actively entering into the fermentation process.
As early as 1962, Le Minor and Ben Hamida had demonstrated the advantages of detection of β-galactosidase over that of lactose fermentation for determining the bacteriological identity of enterobacteria. Slow lactose or lactose negative strains are known to exist within the coliform genera & species. Traditional media ignore these β-galactosidase-positive but permease-negative biotypes. In 1989, Leclerc & Mossel proposed that the presence of β-galactosidase with coliforms be used as the main criteria for classification. The use of a synthetic chromogenic substrate, insensitive to variations in the permeability of lactose, allows the use of this enzyme by a colorimetric reaction.
Buehler *et al.,* in 1949, was the first to identify the presence of a β-D-glucuronidase with *Escherichia coli*. Since then, numerous studies have demonstrated that 94 to 97% of *Escherichia coli* possess a β-D-glucuronidase activity and that the same activity is only rarely encountered with other species (enzyme activity has been detected in a small number of strains of *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella* and in *Yersinia*).

3 PRINCIPLES

The simultaneous presence of two chromogenic substrates allow the detection of two types of specific enzymatic activity : β-galactosidase and β-glucuronidase (GUD).

Microorganisms	Typical phenotype	Colony color
Escherichia coli	GUD $^{+}$ / β-gal $^{+}$	Blue to violet
Non-*Escherichia coli* coliforms	GUD $^{-}$ / β-gal $^{+}$	Pink
Other Gram negative bacteria	GUD $^{-}$ / β-gal $^{-}$	White

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Peptones ..... 18,40 g
- Buffering system ..... 5,80 g
- Growth activators ..... 3,55 g
- Chromogenic mixture ..... 0,44 g
- Selective agents ..... 1,61 g
- Bacteriological agar ..... 11,00 g

pH of the ready-to-use media at 25 °C : 6,9 ± 0,2.

5 PREPARATION

- Dissolve 40,8 g of dehydrated media (BK202) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Distribute into vials or tubes.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain at 44-47 °C.

✓ **Reconstitution :**
40,8 g/L

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

- Deposit 1 mL of the initial sample and its serial dilutions into the bottom of sterile, empty Petri plates.
- Pour roughly 15 mL of molten media per plate.
- Mix well by swirling on a cool, flat surface.
- Incubate at 37 ± 1 °C for 21 ± 3 hours for the enumeration of *Escherichia coli* and total coliforms.
- Incubate at 44 ± 1 °C for 21 ± 3 heures for the enumeration of *Escherichia coli* and thermotolerant (fecal) coliforms.

✓ **Inoculation :**
1 mL in pour plates

✓ **Incubation :**
21 h at 37 °C or 44 °C

Note
In the case of products heavily contaminated in interfering microflora, it is recommended to use a pour plate inoculation in a double layer in order to facilitate the reading.

7 RESULTS

Count the number of colonies on plates containing less than 300 colonies.
Coliforms other than *Escherichia coli* present pink colonies.
Colonies of *Escherichia coli* are blue to violet and may sometimes exhibit a diffuse pink halo around the colonies.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream powder, free-flowing and homogeneous.
Prepared media : amber agar, slightly opalescent.

Typical culture response after 21 h of incubation at 37 °C :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Escherichia coli	WDCM 00013	$P_R \geq 50$ %	Blue to violet colonies
Escherichia coli	WDCM 00012	$P_R \geq 50$ %	Blue to violet colonies
Enterobacter aerogenes	WDCM 00175	$P_R \geq 50$ %	Pink colonies
Enterococcus faecalis	WDCM 00087	Inhibited	-
Staphylococcus aureus	WDCM 00034	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials or tubes (*) : 90 days at 2-8 °C, **shielded from light**.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions..

10 PACKAGING

Dehydrated media :
500 g bottle BK202HA

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

COMPASS® is a registered trademark of SOLABIA S.A.S.

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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Document code : COMPASS ECC_ENv2
Creation date : 07-2010
Updated : 03-2016
Origin of revision : General update.

ANNEX 1 : PHOTO SUPPORT

COMPASS® *Ecc* Agar

Enumeration of *Escherichia coli* and other coliform bacteria.

Results :

Growth obtained after 24 hours of incubation at 37 °C.

Escherichia coli

Characteristic colony : blue-violet color sometimes exhibiting a diffuse pink halo around the colonies.

Coliforms

Characteristic colony : pink color