

Bifidobacterium Agar

Selective medium for the isolation of bifidobacteria from clinical specimens and other materials.

DESCRIPTION

Bifidobacterium Agar is a partially selective medium used for the isolation of *Bifidobacterium* spp. in human stool specimens.

This medium allows good recovery of *Bifidobacterium* species present in the human intestinal tract as well as those strains that can be found in environmental samples and food such as dairy products.

TYPICAL FORMULA (per liter of water)	
Columbia Agar Base	42.5 g
Glucose	2.5 g
Lactulose	2.5 g
Cystein-HCl	0.5 g
Riboflavin	0.01 g
Propionic Acid	5.0 ml
Agar	15.0 g
Final pH 5.5 \pm 0.2 at 25°C	

METHOD PRINCIPLE

The medium is based on Columbia Agar providing amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Glucose is added to accelerate initial growth. Lactulose is a carbohydrate used as prebiotic preferably by bifidobacteria. Cystein is a reducing agent. Riboflavin is a vitamin included to promote growth. Propionic acid is the selective agent inhibiting fungi and many bacteria other than bifidobacteria, such as intestinal *Bacteroides* and *Enterobacteriaceae*. The low pH of the medium further contributes to inhibit other predominating organisms of human feces, such as *Bacteroides* and *Eubacterium* species. Agar is the solidifying agent.

TEST PROCEDURE

Inoculate Bifidobacterium Agar plates by spreading the sample on the agar surface. Incubate anaerobically at 35 ± 2 °C for 2 to 3 days.

NOTES:

- Before use, Bifidobacterium Agar may be kept in an anaerobic atmosphere at room temperature for at least 24 h to increase the recovery of the organisms to be detected.
- Fresh human stool specimens should be suspended in sterile saline or anaerobic saline (saline containing 0.1 g/l cystein-HCl), followed by ten-fold dilutions in the same suspension medium.
- Stool specimens (ideally 10 to 15 grams of stool) must not be older than 24 hours. The use of an anaerobic transport medium is recommended.
- The GasPak EZ Anaerobe systems (ref. 96724, 96726 or 96730) may be used to produce the atmosphere suitable to support the isolation and cultivation of anaerobic bacteria.

INTERPRETING RESULTS

After the incubation, examine plates for growth. Observe colonies microscopically (Gram stains) for the presence of typical bifid, gram positive rods. Count all appropriate colonies of bifidobacteria and multiply by the dilution factor of the sample to obtain the CFU per gram or ml of sample.

NOTES:

- Organisms other than *Bifidobacterium* spp. may grow on this medium.
- Subcultures and biochemical tests must be performed for a final identification of the organisms isolated.
- There exist bifidobacteria that are extremely fastidious and do not grow sufficiently on this or other selective media. Therefore, a non-selective medium, such as Schaedler Agar with Vitamin K1 and 5% Sheep Blood (ref. 11065) should be inoculated in parallel to the selective medium.
- In the feces of healthy individuals, bifidobacteria shall be present in high counts while their absence or low counts may be a hint for intestinal disorder.

APPEARANCE

Slightly opalescent, amber.

STORAGE

Store at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Ready-to-use plates: 6 months.

QUALITY CONTROL

Plates are inoculated with the microbial strains indicated in the QC table. Inoculum for productivity: 50-100 CFU. Inoculum for selectivity: 10^3-10^4 CFU. Incubation conditions: 24-72 h at $35 \pm 2^{\circ}$ C in anaerobic atmosphere.

QC Table.

Microorganism		Specification		
Bifidobacterium animalis	ATCC [®] 25527	Good growth, creamy white colonies, acid odor		
Lactobacillus lactis	ATCC® 19435	Partial to complete inhibition		
Bacterioides fragilis	ATCC [®] 25285	Partial to complete inhibition		
Escherichia coli	ATCC [®] 25922	Partial to complete inhibition		

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for professional use only and must be used by properly trained operators.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

- 1. Gorbach, S.L. 2002. Probiotics in the third millenium. Dig. Liver Dis. 34 (suppl. 2):S2-S7.
- 2. Nebra, Y., and A.R. Blanch. 1999. A new selective medium for *Bifidobacterium* spp. Appl. Env. Microbiol. 65: 5173-5176.
- 3. Hartemink, R., and F.M Rombouts. 1999. Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples. J. Microbiol. Meth. 36: 181-192.
- 4. Kitajima, H., et al. 1997. Early administration of *Bifidobacterium breve* to preterm infants: randomized controlled trial.. Arch. Dis. Child. Fetal Neonatal Med. 76: F101-F107.
- 5. Hartemink, R. et al. 1996. Raffinose-Bifidobacterium (RB) agar, a new selective medium for bifidobacteria. J. Microbiol. Meth. 27: 33-43.
- Silvi, S. et al. 1996. An assessment of three selective media for bifidobacteria in faeces. J. Appl. Bacteriol. 81: 561-564.
 Dave, R.I., and N.P. Shah. 1995. Evaluation of media for selective enumeration of *Streptococcus thermophilus*,
- Barc, R.I., and H.I. Shall. 1995. Evaluation of media for science endineration of successes incliniophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and bifidobacteria. J. Dairy Sci. 79: 1529-1536.
 Beerens, H. 1990. An elective and selective isolation medium for *Bifidobacterium* spp. Lett. Appl. Microbiol. 11:
- 155-157.
- 9. Munoa, F.J., and R. Pares. 1988. Selective medium for isolation and enumeration of *Bifidobacterium* species. Appl. Env. Microbiol. 54: 1715-1718.

PRESENTATION	Format	Packaging	Ref.
Bifidobacterium Agar	90 mm plate	20 plates	10458

TABLE OF SYMBOLS

- 62							
	LOT Batch code	IVD In Vitro Diagnostic Medical Device	Keep away from sunlight		Manufacturer	Fragile, handle with care	Use by
	REF Catalogue number	Do not reuse	Temperature limitation	\sum	Contains sufficient for <n> tests</n>	Caution, consult Instruction For Use	



LIOFILCHEM[®] s.r.l.

Via Scozia zona ind.le, 64026 Roseto degli Abruzzi (Te) Italy Tel. +39 0858930745 Fax +39 0858930330 www.liofilchem.com

