43314 TOS-propionate agar medium acc. ISO (Transgalctosylated oligiosaccharide agar medium; TOS-MUP Agar (Base); Bifidobacteria Selective Count Agar Base; BSC Propionate Agar Base)

TOS-propionate agar medium is recommended for enumeration of presumptive Bifidobacteria by colony count technique from milk products.

Composition:

Ingredients	Grams/Litre
Casein enzymic hydrolysate	10.0
Yeast extract	1.0
Potassium dihydrogen phosphate	3.0
Dipotassium hydrogen phosphate	4.8
Ammonium sulphate	3.0
Magnesium sulphate heptahydrate	0.2
L-Cysteine hydrochloride, monohydrate	0.5
Sodium propionate	15.0
Galactooligosaccharide	10.0
Agar	15.0
Final pH (at 25°C) 6.3 +/- 0.2	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Appearance:	Faintly yellow to brown coloured, homogeneous, free flowing powder.
Gelling:	Firm
Color and Clarity:	Slightly yellow coloured, opalescent gel.

Directions:
Suspend 62.02 grams of dehydrated media in 950 ml distilled water (1 liter for non-selective media). Heat to boiling to dissolvethe medium completely. Sterilize by autoclaving at 115°C for 15 minutes.
For selective isolation of Bifidobacteria add contents of 2 x 25 ml vials of Lithium mupirocin Supplement (69732). Mix carefully to avoid the formation of air bubbles and dispense as desired. Note: This medium being sensitive to heat, excessive heat treatment may therefore indicatively influence the properties of the medium. For more selectivity Glacial acetic acid (Bifido Selective Supplement B, 90577) may also be added. After addition of Bifido Selective Supplement B pH of the medium will shift to the acidic side, which does not affect the performance of the medium.

Test Procedure:
Dried milk products (e.g. infant formula):
Mix the content of a closed sample pack if possible or use a sterile spatula to get a homogenous sample. Weigh 90g of diluent (Quarter-strength Ringer solution) in each of the 250 ml pre-sterilized bottles. Add 10g of the test sample into the bottle with the diluent at 45°C. To dissolve the test sample, swirl slowly. Place in the water bath (45°C) while shaking occasionally. Cool down under running tape water for 2 minutes. Keep all suspension for 30 minutes in the fridge and start directly with the examination.

Yoghurt and yoghurt-like products:
Mix the content of a closed sample pack if possible or use a sterile spatula to get a homogenous sample. Weigh 90g of diluent (Quarter-strength Ringer solution) in each of the 250 ml pre-sterilized bottles. Add 10g of the test sample into the bottle with the diluent at 45°C. To dissolve the test sample, shake and start with the examination as fast as possible

Examination:
Do 1:10 dilution steps with dilution solution until a concentration of 200-500 cfu/ml is obtained. Transfer 1 ml of the appropriate dilutions into a petri dish and add 12-15 ml medium (in duplicates). Mix gently and let solidify. The time between ending the preparation of the primary dilution until addition of culture medium shall not exceed 15 min.

Imediately after solidification of the medium, invert all Petri dishes in the anaerobic culture jar or anaerobic incubator at 37°C for 72 hrs ± 3 hrs. Count the colonies after incubation. Bifidobacterial colonies are recognized by their whitish colour and acetic acid odour. Some of the bifidobacterial strains may appear in different colony size as well as colony appearance on the same plate (1). Select typical colonies for microscopic examination.
Optional a F6PPK-assay can be performed to confirm the result.

Principle and Interpretation:
The medium is specifically prepared for selective enumeration of Bifidobacteria in dairy products where a mixed flora with lactic acid bacteria is present. Casein hydrolysate and yeast extract provide essential sources of nitrogen and other growth nutrients like vitamin B complex. The medium contains a highly purified Galactooligosaccharides, which is one of the most excellent Bifidobacteria growth promoting substances. As well sodium propionate is used to promote the growth of Bifidobacter. Cysteine hydrochloride helps in creating reduced conditions required for the growth of anaerobic Bifidobacteria. Ammonium sulphate is a source of sulfur and nitrogen and magnesium sulphate provides an important divalent cation and sulphate.
Lithium mupirocin is the antimicrobial supplement inhibits the growth of most lactic acid bacteria commonly used in fermented and non-fermented dairy products. Freshly prepared culture media should not be exposed to direct sunlight(1). The potassium phosphates are buffering agents and stabilize the pH.

Cultural characteristics after 48-72 h at 35-37°C (Inoculum: 50-100 cfu) under anaerobic conditions.

Organisms (ATCC)	Growth	Growth with Mupirocin
Bifidobacterium breve (15700)	+++	++/+++
Bifidobacterium infantis (15697)	+++	++/+++
Bifidobacterium longum (15707)	+++	++/+++
Lactococcus lactis (19435)	++/+++	-
Lactococcus cremoris (19257	++/+++	-
Lactobacillus acidophilus (4356)	++	-/+

References:

1. ISO/DIS 29981 IDF 220, Milk products- Enumeration of presumptive bifidobacteria- colony count technique at 37°C, 2010.
2. S.N. Thitaram, G.R. Siragusa, A. Hinton Jr, Letters in Applied Microbiology, vol 41, 355-360, Bifidobacterium selective isolation and enumeration from chiken ceca by an oligosaccharide- antibiotic selective agar medium (2005)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.