

Certificate of Analysis for HM-229

Bacillus smithii, Strain 7_3_47FAA

Catalog No. HM-229

Date: 11 MAR 2013

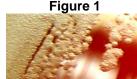
Product Description: Bacillus smithii (B. smithii), strain 7_3_47FAA was isolated in 2007 from an inflamed sigmoid colon biopsy specimen taken from a 25-year-old female patient with Crohn's disease in Calgary, Alberta, Canada.

Lot^{1,2}: 60574083 Manufacturing Date: 15APR2012

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ³	Report results Report results	Gram-positive rod Irregular, flat and cream (Figure 1)
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1460 base pairs)	≥ 99% identical to GenBank: ACWF01000002 (<i>B. smithii</i> , strain 7_3_47FAA)	≥ 99% identical to GenBank: ACWF01000002 (<i>B. smithii</i> , strain 7_3_47FAA)
Viability (post-freeze) ³	Growth	Growth

Quality control of HMP material is only performed to demonstrate that the material distributed by BEI Resources is identical to the deposited material. It should not be considered a complete characterization of the deposited organism.

³72 hours at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy Agar with 5% defibrinated sheep blood



Signature:

Title: Technical Manager, BEI Authentication or designee

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²B. smithii, strain 7_3_47FAA was deposited by Professor Emma Allen-Vercoe, Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada. The deposited material was inoculated into M2GSC medium and grown at 37°C for 6 days in an anaerobic atmosphere (80% N₂:10% CO₂:10% H₂). Optimal culture conditions were determined over the course of 9 additional passages at 37°C, at which time the culture was determined to be impure. Three successive colony purifications were carried out on Tryptic Soy Agar with 5% defibrinated sheep blood at 37°C in an anaerobic atmosphere to obtain a pure culture. The purified culture was found to be facultatively aerobic and was inoculated onto Tryptic Soy Agar with 5% defibrinated sheep blood and incubated for 9 days at 37°C in an aerobic atmosphere with 5% CO₂. The resulting material was then added to Kolles, which were incubated for 72 hours at 37°C in an aerobic atmosphere with 5% CO₂. After an addition passage, the material was harvested to produce this lot.