

Certificate of Analysis for HM-411

Bifidobacterium breve, Strain EX336960VC18

Catalog No. HM-411

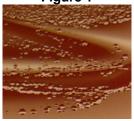
Product Description: *Bifidobacterium breve* (*B. breve*), strain EX336960VC18 was isolated from a human mid-vaginal wall in March 2010 in Richmond, Virginia.

Lot^{1,2}: 59994678 Manufacturing Date: 14SEP2011

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Report results	Gram-positive rod
Colony morphology (anaerobic) ^{3,4}	Report results	Circular, low convex, entire, opaque and light gray (Figure 1)
Colony morphology (aerobic) ^{4,5}	Report results	Circular, low convex, entire, gray
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1420 base pairs)	Consistent with B. breve	Consistent with <i>B. breve</i> ⁶
Viability (post-freeze) ³	Growth	Growth

¹Quality 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.

Figure 1



Date: 06 MAR 2012 Signature: Jack onch

Title: Technical Manager, BEI Authentication or designee

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²B. breve, strain EX336960VC18 was deposited by Professor Gregory A. Buck, Director, Center for the Study of Biological Complexity, Department of Microbiology and Immunology, Virginia Commonwealth University Medical Center, Richmond, Virginia. HM-411 was produced by inoculation of the deposited material into Reinforced Clostridial Broth (<u>ATCC medium 1053</u>) and incubated for 72 hours at 37°C in an anaerobic atmosphere (80% N₂:10% CO₂:10% H₂). The material from the initial growth was passaged once in Reinforced Clostridial Medium for 48 hours at 37°C in an anaerobic atmosphere to produce this lot.

³48 hours at 37°C in an anaerobic atmosphere on Tryptic Soy Agar with 5% defibrinated sheep blood

⁴Anaerobic and aerobic colony types were observed when HM-⁴11 was grown on Tryptic Soy Agar with 5% defibrinated sheep blood, for 48 hours. The 16S gene of each colony type was sequenced and both anaerobic and aerobic colonies were consistent with *B. breve*. *B. breve* is a facultative anaerobe and the presence of aerobic growth is not unexpected.

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

⁶The sequence obtained for HM-411 could only be compared with published sequences. No 16S sequence was provided by the depositor, therefore, a direct comparison could not be performed.



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