

Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-Aug-2022
Document Type: Dietary Supplement Monographs
DocId: GUID-06136661-8490-48DD-B094-D0844E4CA85C_2_en-US
DOI: https://doi.org/10.31003/USPNF_M14375_02_01
DOI Ref: cqw45

© 2025 USPC
Do not distribute

Add the following:

▲**Bifidobacterium longum subsp. infantis**

DEFINITION

Bifidobacterium longum subsp. *infantis* comprises Gram-positive, non-spore forming, lactic acid-producing anaerobic, facultative hexose heterofermenters. They have various shapes, including short, curved rods, club-shaped rods, and bifurcated Y-shaped rods. Suitable cryoprotectants may be added to the concentrated bacteria following fermentation, after which the product is frozen and freeze-dried. The formulated product may be blended with suitable diluents and/or bulking agents. It contains NLT 100% of the labeled viable cell count of the specified strain of *Bifidobacterium longum* subsp. *infantis*.

- **Strain Bi-26:***Bifidobacterium longum* subsp. *infantis* Bi-26 (ATCC SD-6720) is a pure, specific strain of *Bifidobacterium longum* subsp. *infantis*. It is a non-D-lactate producer.

[NOTE—The nucleic acid-based method detailed in *Identification B* only applies to *Bifidobacterium longum* subsp. *infantis* Bi-26.]

IDENTIFICATION

• **A. MICROSCOPIC IDENTIFICATION**

Analysis: Proceed as directed in the Assay and perform microscopic examination.

Acceptance criteria: Bacilli-shaped including short, curved rods, club-shaped rods, and bifurcated Y-shaped rods.

• **B. NUCLEIC ACID-BASED IDENTIFICATION**

[NOTE—In all cases for *Identification B*, “sterile water” refers to sterile, nuclease-free water acceptable for use in molecular biology.¹]

Buffer: Use a molecular biology-grade 10 mM tris-hydrochloride, 1 mM EDTA sodium buffer.²

Sample solution: 100 mg/mL of the freeze-dried probiotic powder in *Buffer*

Primer set 1: Use a primer set comprised of forward primer sequence (5'–3') GTCACGATGTCTCCTTTGATATCAGCATG and reverse primer sequence (5'–3') CCTTTTGGCTCTCCCCG.³ Dilute primers in *Buffer* to a stock concentration of 100 μM, further dilute in *Buffer* to 25 μM, and store at –20°.

Primer set 2: Use a primer set comprised of forward primer sequence (5'–3') TCGCCGACATGCGAGACGAA and reverse primer sequence (5'–3') TCCAGGCTCAGCGTGCGA.³ Dilute primers in *Buffer* to a stock concentration of 100 μM, further dilute in *Buffer* to 25 μM, and store at –20°.

Polymerase chain reaction (PCR) sample preparation: For each primer set, prepare a *PCR sample preparation* containing 1 μL of the *Sample solution*, 12.0 μL of mastermix polymerase,⁴ 1 μL of diluted forward primer (25 μM), 1 μL of diluted reverse primer (25 μM), and 10 μL of sterile water.

PCR negative control: Prepare as directed for the *PCR sample preparation*, except replace the *Sample solution* with 1 μL of sterile water.

PCR positive control: Prepare as directed for the *PCR sample preparation*, except replace either the *Sample solution* with 1 μL of the genomic DNA of the reference strain or the strain-specific primers with species-specific universal primers for *Bifidobacterium longum* subsp. *infantis*.

PCR amplification: Perform PCR on each *PCR sample preparation*, the *PCR negative control*, and the *PCR positive control* using an appropriate thermal cycler.⁵ Perform the PCR amplification cycle per primer as specified in [Table 1](#).

Table 1. PCR Amplification Cycle

Primer Set	PCR Amplification Cycle
Primer set 1	Incubate at 95° for 7 min (step 1), 95° for 30 s (step 2), 59° for 30 s (step 3), and 72° for 1 min (step 4). Repeat steps 2–4 for 34 cycles, then incubate at 72° for 5 min, and hold at 4°.
Primer set 2	Incubate at 95° for 7 min (step 1), 95° for 30 s (step 2), 61° for 30 s (step 3), and 72° for 1 min (step 4). Repeat steps 2–4 for 34 cycles, then incubate at 72° for 5 min, and hold at 4°.

Analysis: Analyze the products of the *PCR amplification* for each *PCR sample preparation*, the *PCR positive control*, and the *PCR negative control* by using an automated on-chip electrophoresis system with a DNA kit.⁶ Follow the manufacturer's instructions for analysis.

Alternatively, analysis and visualization may be accomplished using gel electrophoresis. Prepare or use a commercially available 2% (w/v) agarose gel in a 1X tris-acetic acid–EDTA buffer (40 mM tris-hydrochloride, 1% glacial acetic acid, and 1 mM EDTA). Stain the gel with 0.5 mg/mL of ethidium bromide in water and de-stain with deionized water. [CAUTION—Ethidium bromide is considered a toxic substance and a potential mutagen. Use appropriate personal protective equipment (including nitrile gloves) when handling this reagent.] Use a DNA ladder standard (1 KB plus)⁷ suitable for determining the size of linear double-stranded DNA fragments amplified by the appropriate *Primer set*. The ladder standard should be used in the first and last lanes on the gel to allow for proper comparison of amplicons.

Analysis of *PCR positive control* must result in the amplification of specific base pairs as indicated in the acceptance criteria if using genomic DNA of the strain or must result in the amplification of specific base pairs as found in the published literature if using species-specific universal primers. Analysis of the *PCR negative control* must result in the absence of any amplification products. If the results are not as expected, preparation of the *PCR sample preparation*, the *PCR positive control*, and the *PCR negative control* must be repeated, followed by *PCR amplification and Analysis*.

Acceptance criteria

Primer set 1: The *PCR sample preparation* gives an amplification product of 105 base pairs.

Primer set 2: The *PCR sample preparation* gives an amplification product of 181 base pairs.

ASSAY

• ENUMERATION

(Follow [Probiotic Tests \(64\)](#), [Enumeration](#), [Enumeration for Non-Spore-Forming Bacteria Strains](#) with the following exceptions.)

Lactobacilli MRS agar medium: Prepare *Lactobacilli MRS agar medium* as directed in the chapter with the addition of 1 mL of sterile 5% (w/v) cysteine hydrochloride solution to each 100 mL for a final cysteine hydrochloride concentration of 0.05% immediately before use.

Buffered peptone water: Prepare according to [Table 2](#) or use a suitable commercially available broth.⁸

Table 2. Buffered Peptone Water

Reagent	Quantity (g)
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Monopotassium phosphate	1.5
pH	7.2 ± 0.2

Suspend *Buffered peptone water* in 1 L of purified water in an appropriately sized conical flask or beaker. Cover the flask or the beaker with aluminum foil and heat to boiling with stirring on a hot plate. Allow to boil for 1 min to completely dissolve the broth ingredients, and then autoclave the solution at 121° for 15 min. Broth may also be aseptically transferred into individual media bottles in 100- or 200-mL aliquots before sterilizing and then autoclaved and stored for later use. The broth can be stored at a temperature between 2° and 8° in a sterile well-closed container unless it is intended for immediate use. Do not use the medium for longer storage period than has been validated. Allow broth to come to room temperature before use.

Sample preparation: Use *Buffered peptone water* instead of *Lactobacilli MRS broth* and follow all steps as directed in the chapter.

Acceptance criteria: NLT 100% of the labeled viable cell count, in cfu/g, of the specified strain of *Bifidobacterium longum* subsp. *infantis*.

CONTAMINANTS

[NOTE—The methods of microbial analysis included in this section as examples represent currently accepted methods commonly used in industry. Users may substitute other validated test methods for the methods in this section.]

• **PROBIOTIC TESTS (64), Contaminants, Contaminant Microorganisms:** The total combined molds and yeasts count does not exceed 10² cfu/g. The total non-lactic acid bacteria count is less than 5 × 10³ cfu/g.

• **PROBIOTIC TESTS (64), Contaminants, Specified Microorganisms:** It meets the requirements of the test for absence of *Escherichia coli* in 10 g. It meets the requirements of the test for absence of *Salmonella* species in 40 g.

• **LISTERIA:** (See *Food Chemicals Codex*, Appendix XV.) It meets the requirements of the test for absence of *Listeria* in 25 g.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in high barrier foil laminate bags and store at or below 4°.

• **LABELING:** This ingredient should be labeled with the genus, species, and strain names and with the formulated enumeration in cfu/g or cfu/dose. This monograph applies only to *Bifidobacterium longum* subsp. *infantis* Bi-26.▲ (USP 1-Aug-2022)

¹ Suitable PCR-Certified Waters, RNase and DNase Free, are available from www.teknova.com.