Cat. # NN0002

For Research Use

TaKaRa

16S DNA Quantitative Standard for Microbiome

Product Manual

v202511w

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I. Description

This product is a synthetic DNA standard that can be used for quantitative 16S rRNA microbiota analysis using next-generation sequencing (NGS). This standard consists of 12 types of DNA with artificially designed 16S rRNA genes (rDNA), variable regions (V1 to V9), and mixed-in predetermined copy numbers. Because these sequences do not exist in nature, adding them as a spike-in control to an analytical sample does not affect experimental results from microbiota or other biological samples (Figure 1). This product is spiked into the DNA sample for analysis and 16S rRNA NGS analysis is performed. A standard curve is then created by plotting the theoretical 16S rDNA copy number for each standard sequence on the X-axis and the number of reads obtained for each standard sequence on the Y-axis. This enables absolute quantification of bacterial samples, which was previously difficult with conventional 16S rRNA NGS analysis (Table 1). In addition, this product can be used as a control to evaluate the performance of assay designs and to control the accuracy of each assay.

The artificial sequences in this product include regions V1 to V9, making it compatible with various 16S rRNA NGS analysis methods.

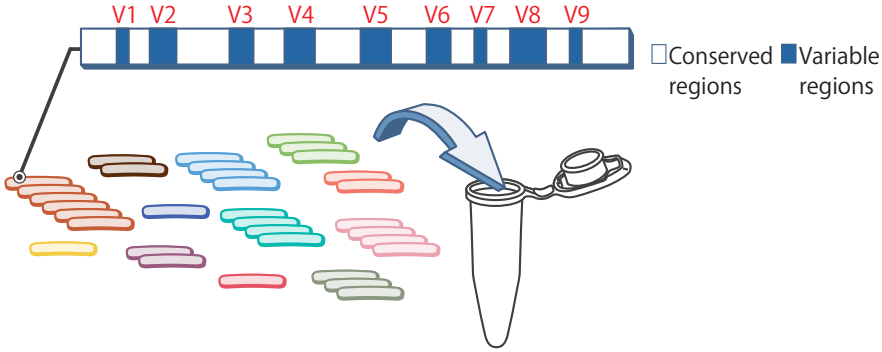

Figure 1. Mixture of 12 different 16S rDNA sequences with different copy numbers.

The variable regions consist of artificially designed sequences, and the conserved regions are from the 16S rDNA sequence. Therefore, by designing primers in the conserved regions, it is possible to simultaneously amplify the 16S rDNA sequence of the bacterium and the 16S rDNA sequence of this standard.

Table 1. Composition of the undiluted 16S DNA quantitative standard (1.3 x 10^8 copies/μl)

Identifier	GenBank accession number	16S rDNA (copies/μl)	16S rDNA copies (%)
Std_01	LC140931	6.31 x 10^7	47.39
Std_02	LC140932	3.32 x 10^7	24.93
Std_03	LC140933	1.75 x 10^7	13.14
Std_04	LC140934	9.20 x 10^6	6.91
Std_05	LC140935	4.84 x 10^6	3.63
Std_06	LC140936	2.55 x 10^6	1.91
Std_07	LC140937	1.34 x 10^6	1.01
Std_08	LC140938	7.06 x 10^5	0.53
Std_09	LC140939	3.72 x 10^5	0.28
Std_10	LC140940	1.96 x 10^5	0.15
Std_11	LC140941	1.03 x 10^5	0.08
Std_12	LC140942	5.42 x 10^4	0.04

II. Components (200 reactions)

◯ 16S DNA Quantitative Standard	1.3 x 10^8 copies/ μl	20 μl
16S DNA Dilution Buffer		300 μl

III. Storage -20℃

IV. Materials Required but not Provided

【Reagents】

- Nucleic acid extraction kit
  [For stool samples]
  NucleoSpin DNA Stool (Cat. #740472.10/.50/.250)* etc.
  [For soil samples]
  NucleoSpin Soil (Cat. #740780.10/.50/.250)* etc.
- Purification of PCR products
  NucleoMag NGS Clean-up and Size Select (Cat. #744970.5/.50/.500)* etc.
  80% Ethanol
  Eluate (10 mM Tris-HCl, pH 8.5)
- 16S rRNA Sequence Library preparation kit

【Instruments】

- 0.2 ml magnetic stand
- Micropipettes and tips
- PCR tubes etc.

【Equipment】

- Thermal cycler
  Clontech PCR Thermal Cycler GP (Cat. #WN400)* etc.
- High speed microcentrifuge

* Not available in all geographic locations. Check for availability in your area.

V. Protocol

【Note】 The standard curve is designed to include many bacteria by mixing 1.3 x 10^7 copies of 16S DNA Quantitative Standard with 10 ng of DNA sample. If the DNA sample contains a large amount of host genome, etc., and the results deviate from the standard curve, increase or otherwise adjust the amount of DNA sample used.

V-1. Preparation of 10-fold Diluted 16S DNA Quantitative Standard (1.3 x 10^7 copies/μl)

Dilute the 16S DNA Quantitative Standard 10-fold with 16S DNA Dilution Buffer.

【Note】 • Prepare DNA diluents just before use.
• Prepare the kit using 5 μl or more of the undiluted 16S DNA Quantitative Standard (1.3 x 10^8 copies/μl).

[Preparation example]
1. Dispense 45 μl of 16S DNA Dilution Buffer into a 1.5 ml microtube.
2. Add 5 μl of 16S DNA Quantitative Standard (1.3 x 10^8 copies/μl), mix well, and spin down.

V-2. Sample Preparation

Prepare a 16S rRNA sequence library using 10 ng of DNA extracted from the specimen and 1 μl of the 10-fold diluted 16S DNA quantitative standard (1.3 x 10^7 copies/μl) prepared in Step V-1 as a template.

【Note】 Follow the instructions for each reagent when preparing the 16S rRNA Sequence Library.

VI. Analysis

1. Construct OTUs (Operational Taxonomy Units) from the acquired sequence reads.
2. Extract representative sequences from the constructed OTUs, and subject the OTUs to microbiota phylogenetic classification.
3. Obtain read counts for the phylogenetically classified microbiota and synthetic 16S rDNA standards.
4. Create a standard curve by plotting the theoretical 16S rDNA copy number for each standard sequence on the X-axis and the number of reads obtained for each standard sequence on the Y-axis, and calculate the 16S rDNA copy number from the number of reads for each bacterium (Table 2).

Table 2. Composition of the 10-fold diluted 16S DNA quantitative standard (1.3×10^7 copies/μl)

Identifier	GenBank accession number	16S rDNA (copies/μl)	16S rDNA copies (%)
Std_01	LC140931	6.31×10^6	47.39
Std_02	LC140932	3.32×10^6	24.93
Std_03	LC140933	1.75×10^6	13.14
Std_04	LC140934	9.20×10^5	6.91
Std_05	LC140935	4.84×10^5	3.63
Std_06	LC140936	2.55×10^5	1.91
Std_07	LC140937	1.34×10^5	1.01
Std_08	LC140938	7.06×10^4	0.53
Std_09	LC140939	3.72×10^4	0.28
Std_10	LC140940	1.96×10^4	0.15
Std_11	LC140941	1.03×10^4	0.08
Std_12	LC140942	5.42×10^3	0.04

<Standard curve evaluation points>

- The Pearson correlation coefficient between the number of 16S rDNA copies and the number of reads for each 16S rDNA sequence must be $\geqq 0.96$.
- For the standard curve $Y=aX+b$, the coefficient of determination R^2 is > 0.90.

VII. References

1) Klindworth A, *et al*. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res*. 2013 Jan 7, **41**(1): e1.
2) Caporaso JG, *et al*. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* . 2012 Aug, **6**(8): 1621-1624.
3) Tourlousse D, *et al*. Synthetic spike-in standards for high-throughput 16S rRNA gene amplicon sequencing. *Nucleic Acids Res*. 2017 Feb 28; **45**(4): e23.