Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
WHO International Reference Reagent for Gut Microbiome Analysis by Next-Generation Sequencing (DNA-Gut-HiLo)
NIBSC code: 20/304
Instructions for use
(Version 5.0, Dated 26/01/2024)

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1. INTENDED USE

This material is a mix of purified nucleic acids isolated from bacterial strains that reside in the human intestine (see Table 1). This a reference preparation is intended as a control reagent for Next Generation Sequencing analysis of gut microbiome samples, and more broadly to assess the quality of laboartory methods and software analaysis used in these studies.

2. CAUTION

The material is not of human or bovine origin. This preparation is not for administration to humans or animals

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

No unitage is assigned to this material.

4. CONTENTS

Country of origin of biological material: United Kingdom.
0.25 ml of freeze-dried material consisting of DNA from 20 bacterial strains (see Table 1) at a final concentration of between 3-5 ng/µl upon reconstitution with 50 µl.

Table 1

Strain number	Strain name	% content (genome size adjusted)
DSM 22959	Akkermansia muciniphila	0.18
DSM 17242	Alistipes finegoldii	1.3
DSM 3319	Anaerostipes hadrus	1.75
DSM 2079	Bacteroides thetaiotaomicron	7.72
DSM 6597	Bacteroides uniformis	1.05
DSM 20088	Bifidobacterium longum subsp. infantis	17.2
DSM 20219	Bifidobacterium longum subsp. longum	19.82
DSM 19850	Blautia wexlerae	0.11
DSM 10702	Clostridium butyricum	10.59
DSM 13712	Collinsella aerofaciens	1.99
DSM 1103	Escherichia coli	9.33
DSM 3353	Eubacterium/Anaerobutyricum hallii	1.48
DSM 17677	Faecalibacterium praunitzii	0.16
DSM 20077	Lactobacillus paragasserii/gasseri	0.26
DSM 20701	Parabacteroides distasonis	10.1
DSM 18205	Prevotella/Segatella copri	13.84
DSM 7089	Prevotella melaninogenica	1.53
DSM 16839	Roseburia hominis	1.35
DSM 14610	Roseburia intestinalis	0.11
DSM 19829	Ruminococcus gauvreauii	0.12

5. STORAGE

Unopened ampoules should be stored at -20 C.
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Upon receipt, ampoules should be stored at -20 C until use. Prior to use in assays, freeze-dried material are to be reconstituted with 50µl of sterile nuclease-free water. To ensure complete reconstitution of samples it is recommended that samples are gently but thoroughly mixed by pipetting material up and down at least 10 times. Before reconstitution please ensure the whole freeze-dried material is at the bottom of the ampoule, improper reconstitution will result in a reagent of lower concentration. Samples should be reconstituted on the day of the assay.

Users should follow the instructions in https://doi.org/10.1186/s40168-020-00856-3 to calculate the four key reporting measures. The results should comply with the Minimum Quality Criteria (MQC) which were established using data obtained in the WHO collaborative study:

Table 2: Minimum quality criteria of participant shotgun sequencing at the species level

Measure	Sensitivity (%)	FPRA	Diversity	Similarity (%)
Optimal	100	0	19	100
MQC	≥68	≤7.29	13-19	≥68

Table 3: Minimum quality criteria of participant 16S rRNA amplicon sequencing at the genera level

Measure	Sensitivity (%)	FPRA	Diversity	Similarity (%)
Optimal	100	0	16	100
MQC	≥94	≤0.79	16	≥58 ≥49*

*16S copy number adjusted

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

Once reconstituted, any remaining material can be stored at -20°C and is stable for up to 2 freeze-thaw cycles.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

https://doi.org/10.1186/s40168-020-00856-3

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

10. ACKNOWLEDGEMENTS

We would like to express our thanks to SPD (NIBSC) for assistance in the determination of freeze drying conditions and for moisture and oxygen determinations of the ampouled material and the staff of CBRM for assistance with the filling procedure.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	Yes
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	see caution, section 1		

Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Irritating to mouth, throat and stomach, avoid ingestion
Effects of skin absorption:	May cause skin irritation, avoid contact with skint established, avoid contact with skin

Suggested First Aid	
Inhalation:	Move to fresh air and seek medical advice.
Ingestion:	Wash out mouth with water, provided person is conscious and seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water. Seek medical advice if symptoms occur.

Action on Spillage and Method of Disposal
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.
Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.25 - 0.5 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.