



International Ref. Reagent

**WHO International Reference Reagent for Gut Microbiome DNA
Extraction (whole cell)
NIBSC code: 22/210
Instructions for use
(Version 4.0, Dated 21/02/2024)**

This material is not for in vitro diagnostic use

1. INTENDED USE

This material is a mix of inactivated (dead) bacterial strains that reside in the human intestine (see Table 1 for list of strains). This reference preparation is intended for use as a control reagent to evaluate DNA extraction protocols commonly used in microbiome pipelines.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The material is not of human or bovine origin. 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.
Freeze-dried material consisting of inactivated (dead) cells from 20 bacterial strains (see Table 1) at a concentration of 2×10^{10} cells/ml upon reconstitution with 500 μ l of PBS.

Table 1: List of bacterial strains (equal amount of each)

Strain number	Strain name	Gram +/-
DSM 22959	Akkermansia muciniphila	-
DSM 17242	Alistipes finegoldii	-
DSM 3319	Anaerostipes hadrus	+
DSM 2079	Bacteroides thetaiotaomicron	-
DSM 6597	Bacteroides uniformis	-
DSM 20088	Bifidobacterium longum subsp. infantis	-
DSM 20219	Bifidobacterium longum subsp. longum	+
DSM 19850	Blautia wexlerae	+
DSM 10702	Clostridium butyricum	+
DSM 13712	Collinsella aerofaciens	+
DSM 1103	Escherichia coli	-
DSM 3353	Eubacterium/Anaerobutyricum hallii	+
DSM 17677	Faecalibacterium praunitzii	+
DSM 20077	Lactobacillus paragasseri/gasseri	+
DSM 20701	Parabacteroides distasonis	-
DSM 18205	Prevotella/Segatella copri	-
DSM 7089	Prevotella melaninogenica/duncaniae	-
DSM 16839	Roseburia hominis	+
DSM 14610	Roseburia intestinalis	+
DSM 19829	Ruminococcus gauvreauii	+

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 500 μ l of sterile PBS. 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. Samples should be reconstituted on the day of the assay.

Users should follow the instructions in <https://doi.org/10.1186%2Fs40168-022-01313-z> [1] 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 [2]:

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

Measure	Sensitivity (%)	FPRA	Diversity	Similarity (%)
Optimal	100	0	19	100
MQC	≥ 89	0	17-19	≥ 62

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.

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

9. REFERENCES

- <https://doi.org/10.1186%2Fs40168-022-01313-z>
- Anwar S, Mate R, Sinnakandu P et al. 2023. A WHO collaborative study to evaluate the candidate WHO International Reference Reagent for DNA extraction of the Gut Microbiome. WHO Expert Committee on Biological Standardization. WHO/BS/2023.2455

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 2
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 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: 0.5 - 0.75g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No