



**WHO International Standard
1st International Standard 2019 for ATDB102 Reference Genome
NIBSC code: 18/164
Instructions for use
(Version 1.0, Dated 16/03/2020)**

1. INTENDED USE

Material 18/164 is of freeze-dried, purified genomic DNA (gDNA) extracted from ATDB102 human cell line. The material has proven to be wild-type for *PIK3CA* c.1633G>A (E545K), *TP53* c.916C>T (R306*), *NRAS* c.34G>T (G12C), *PTEN* c.795delA (K267fs*9) and *MAP2K1/MEK1* c.199G>A (D67N) variants and to be homozygous wild-type for *PIK3CA*, *TP53*, *NRAS*, *PTEN*, *MAP2K1/MEK1*. The material may be used both as a common reference and diluent for the above variants in the 1st International Standards 2019 for HCT 15 and MOLT-4 Cancer Genomes (NIBSC material codes 18/118 and 18/130). Details on how to use the material as a diluent is provided in the Instructions for Use for material 18/118 and 18/130. The material is intended for use as a primary standard for the calibration of secondary standards, kits, and assays. The material is not intended as a run control. The material was tested by external laboratories and showed suitability as a standard in next-generation sequencing (NGS) and digital PCR (dPCR). The material also comprises non-clinically relevant variants that may be used in the validation of NGS assays (see APPENDIX I). The material was established in 2019 by the Expert Committee on Biological Standardization of the World Health Organization (WHO) as the WHO 1st International Standard for ATDB102 Reference Genome, NIBSC material code 18/164.

2. CAUTION

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

The cell line used in the preparation of this material was tested and found negative for mycoplasma, HIV1, HTLV1, HBV, and HCV by PCR. This cell line is an Epstein Barr virus (EBV)-transformed lymphoblastoid cell line. EBV is a category 2 pathogen as classified by the UK Advisory Committee on Dangerous Pathogens. EBV sequences may be present in these materials, but the DNA has been prepared using a protocol in which proteins are denatured and removed, thus likely inactivating the virus. However, the potential for viable virus to survive cannot be eliminated. 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

The material was tested in an international collaborative study involving 35 laboratories and 38 testing methods. The genotype was obtained from NGS and dPCR (Table 1).

NIBSC material code	Nominal Variant	Consensus variant percentage (%)	Consensus variant copy number per diploid human genome mass	Consensus total copy number per diploid human genome mass
18/164	<i>PIK3CA</i> c.1633G>A (E545K)	0.0	N/A	2
	<i>TP53</i> c.916C>T (R306*)	0.0	N/A	2
	<i>NRAS</i> c.34G>T (G12C)	0.0	N/A	2
	<i>PTEN</i> c.795delA (K267fs*9)	0.0	N/A	2
	<i>MAP2K1/MEK1</i> c.199G>A (D67N)	0.0	N/A	2

Table 1. Consensus values for the WHO 1st International Standard 2019 for ATDB102 Reference Genome (NIBSC material code 18/164).

4. CONTENTS

Country of origin of biological material: United Kingdom.

The coded ampoule contains approximately 5µg freeze-dried, purified genomic DNA extracted from ATDB102 human cell line. The gDNA was extracted using a 'salting out' method, and diluted in Tris-EDTA buffer with 5mg/ml Trehalose before freeze-drying.

5. STORAGE

Store all unopened ampoules of the freeze-dried materials at -20°C or below. **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

- Open the ampoule as described in section 6, above.
- Reconstitute the freeze-dried material at room temperature with 100µl nuclease-free water.
- Transfer the sample to a nuclease-free tube using a pipette, ensuring the maximum available volume is collected.
- Allow the material to reconstitute for 1 hour at room temperature and pipette well to mix. The DNA concentration will now be approximately 50ng/µl in 1x Tris-EDTA buffer but confirmation with own quantification method is recommended before use. The possible appearance of white flecks in the material should not be of concern.
- This material may be used as a wild-type control and as preferable diluent to produce standards at any chosen variant percentages for material 18/118 and 18/130 (1st International Standards 2019 for HCT 15 and MOLT-4



Cancer Genomes); see relevant Instructions for Use for details on how to use it as diluent. If insufficient material 18/164 is available to perform the dilutions, an alternative wild-type gDNA may be aligned to material 18/164 and used as diluent i.e. should be confirmed as being diploid, wild-type for *PIK3CA* c.1633G>A (E545K), *TP53* c.916C>T (R306*), *NRAS* c.34G>T (G12C), *PTEN* c.795delA (K267fs*9) and *MAP2K1/MEK1* c.199G>A (D67N) variants and homozygous wild-type for *PIK3CA*, *TP53*, *NRAS*, *PTEN*, *MAP2K1/MEK1*.

f. Add the required amount to your assay. Material may be further diluted (with nuclease-free water or suitable buffer) to achieve a DNA concentration appropriate for your assay.

g. Primary and secondary standards should be analysed in the same assay to assign values to the secondary standards. If further information is required, please contact grmteam@nibsc.org.

8. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. It is the policy of the WHO to not assign an expiry date to their international reference materials. They remain valid with the assigned values and status until withdrawn or amended. Reference materials are held at NIBSC within assured, temperature controlled storage facilities. Reference materials should be stored on receipt as indicated on the label. Accelerated degradation studies have indicated that these materials are suitably stable when stored at -20°C or below, for the assigned values to remain valid until the materials are withdrawn or replaced. These studies have also shown that the materials are suitably stable for shipment at ambient temperature without any effect on the assigned values. It is highly recommended that the material is used on the day it is reconstituted and is not stored. However, in-house analysis determined reconstituted freeze-dried genomic DNA to be stable for up to 4 days at +4°C (or 2 months at -20°C). Care should be taken to avoid cross contamination with other samples. Users who have any data supporting any deterioration in the characteristics of materials are encouraged to contact NIBSC.

9. REFERENCES

1. WHO document
http://www.nibsc.org/documents/ifu/SupplementaryInformation/18-164/WHO_BS.2019.2368.pdf

10. ACKNOWLEDGEMENTS

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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: white crystalline solid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	contains material of human origin
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
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: 3.5g per ampoule
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_biologicalstandardsrev2004.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.



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APPENDIX I. ADDITIONAL NON-CLINICALLY RELEVANT VARIANTS

Further information about the non-clinically relevant variants to be used for the validation of NGS assays, can be found at:
<http://www.nibsc.org/documents/ifu/SupplementaryInformation/18-164/Additional01.xlsx>.

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

