Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
1 st International Standard 2019 for MOLT-4 Cancer Genome
NIBSC code: 18/130
Instructions for use
(Version 1.0, Dated 16/03/2020)

1. INTENDED USE

Material 18/130 is of freeze-dried, purified genomic DNA (gDNA) extracted from MOLT-4 human cell line. The material has associated consensus variant percentages for *TP53* c.916C>T (R306*), *NRAS* c.34G>T (G12C), *PTEN* c.795delA (K267fs*9) and *MAP2K1/MEK1* c.199G>A (D67N) variants and consensus variant and total *TP53, NRAS, PTEN, MAP2K1/MEK1* copy numbers per diploid human genome mass. The material may be diluted by application of a calculation (see APPENDIX I) to produce standards at a range of consensus variant percentages for any of the above variants. 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 run control. The material was tested by external laboratories and showed suitability as a standard in next-generation sequencing (NGS) and digital PCR (dPCR). In addition to the above clinically-relevant variants, the material also comprises also non-clinically relevant variants that may be used in the validation of NGS assays (see APPENDIX II). 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 MOLT-4 Cancer Genome, NIBSC material code 18/130.

2. CAUTION

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

The preparation contains material of human origin, which has been tested and found negative for HIV 1 and 2 Ab/Ag testing and HBsAg (Hep b surface antigen) by serology and HCV by NAT (Nucleic Acid Test). 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 and consensus mutation percentage was obtained from NGS and dPCR (Table 1). End-users are able to further dilute the material (with wild-type material 18/164, or another wild-type genomic DNA calibrated to material 18/164) using a dilution formula based on the variant and total gene copy numbers per diploid human genome mass, to achieve further standards at a range of lower variant percentages from which assay calibration may be achieved, see section 7 and APPENDIX I.

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
18/130	*TP53* c.916C>T (R306*)	31.8	0.48327	1.52050
	NRAS c.34G>T (G12C)	24.7	0.45954	1.85863
	PTEN c.795delA (K267fs*9)	100.0*	1.78544	1.78545
	MAP2K1/MEK1 c.199G>A (D67N)	25.3	0.44476	1.75702

Table 1. Consensus values for the WHO 1st International Standard 2019 for MOLT-4 Cancer Genome (NIBSC material code 18/130). Genotype, consensus variant percentage, and consensus copy numbers per diploid human genome mass for use in calculating how the material may be diluted to prepare further standards at lower variant levels, are shown. * *PTEN* c.795delA (K267fs*9) wild-type calculated as 0.00001% but likely to be 0, therefore consensus variant percentage shown as 100.0%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The coded ampoule contains approximately 5µg freeze-dried, purified genomic DNA extracted from human cell lines. 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
a. Open the ampoule as described in section 6, above.
b. Reconstitute the freeze-dried materials at room temperature with 100µl nuclease-free water.
c. Transfer the sample to a nuclease-free tube using a pipette, ensuring the maximum available volume is collected.
d. 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.
e. This variant material may be combined with material 18/164 (wild-type) to produce standards at any chosen variant percentage; see APPENDIX I.

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

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.t

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/18-130/WHO_BS.2019.2368.pdf

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the significant contributions of all collaborative study participants.
Particular thanks go to Simon Patton of EMQN (Manchester, UK) for connecting us with some participants. We would also like to extend our gratitude to Paul Matejtschuk, Sara Jane Holmes, James Condron and the Standardization Science group at NIBSC, along with the Standards Processing Division for their development, and processing of the materials; Dahud Kahan for helping us to set up the dedicated (secure and encrypted) ShareFile Web Page and Sophie McLachlan from the MHRA communications team.

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_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.

APPENDIX I. DILUTION TO GENERATE ADDITIONAL STANDARDS

Material 18/130 may be diluted to produce further standards at lower variant percentages. The preferable diluent is the wild-type material 18/164 (WHO 1st International Standard 2019 for ATDB102). However, 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 the diluent i.e. it should be confirmed as being wild-type, diploid, and containing two copies of the gene of interest per diploid genome mass.

Notes:

- When preparing the dilutions, it is important to calculate the amount of wild-type gDNA needed to carry out all the dilution points;
- A minimum of 5 dilution points (including the crude material) is recommended.

Further details on the dilution response of these materials may be found in the WHO report on the collaborative study to evaluate the proposed WHO 1st International Standards for Cancer Genomes: http://www.nibsc.org/documents/ifu/SupplementaryInformation/18-130/WHO_BS.2019.2368.pdf.

Dilutions of the variants may be established as follows:

1. By use of the formula:

$$dilution\ response = \left(\frac{variant\ copy\ number}{percentage\ of\ variant} * 100 - total\ copy\ number\right) * \frac{1}{2} + 1 \quad (1)$$

where the variant copy number and total copy number per human diploid genome mass can be taken from Table 1.

For example, to prepare a standard of 15% variant percentage for *TP53* c.916C>T (R306*), the allelic content figures are used thus:

$$1.85 = \left(\frac{0.48327}{15} * 100 - 1.52050\right) * \frac{1}{2} + 1 \quad (2)$$

Meaning that a 1 in 1.85 dilution (in blue in example formula 2) of material 18/130 with the wild-type material 18/164 (or another wild-type gDNA aligned to 18/164), will yield a further standard of consensus mutant percentage 15% (in green in example formula 2) *TP53* c.916C>T (R306*), for example, 2.00 µl material 18/130, plus 1.70 µl material 18/164.

Note:

- It is important to use the 5 decimal places for copy numbers in the calculation to achieve a maximally accurate answer.

2. By reference to dilution curves available from NIBSC:

Use Google Chrome to open the link for an interactive dilution curve:
http://www.nibsc.org/documents/ifu/SupplementaryInformation/18-130/InteractiveDilutionCurves.html

For each variant, hover the "+" cursor over the dilution curve at the variant percentage required to see the dilution to be performed.

For example, to prepare a further standard of 15% (in green in example formula 2) variant percentage for *TP53* c.916C>T (R306*), hover the "+" cursor over 15% on the curve to see the dilution required i.e. 1.85 means that a 1 in 1.85 dilution (1 part of material 18/130 plus 0.85 parts of wild-type, material 18/164) will yield a further standard of variant percentage 15% , 2.00 µl material 18/130, plus 1.70 µl material 18/164.

Notes:

- The variant percentage (%) is shown at 5 decimal places to ensure the accuracy of the dilution curves. Users are likely to be working with maximum 1 or 2 decimal places so rounding may be required.
- Performance in other browsers cannot be guaranteed.

3. By use of pre-calculated dilutions:

Refer to Table 2 for details on the preparation of further standards for each nominal variant at a range of variant percentages.

NIBSC material code	Nominal Variant	Consensus variant copy number per diploid human genome mass	Consensus total copy number per diploid human genome mass	Wanted variant %	Dilution to be performed	Volume mutant material (µl)	Volume wild-type material (µl)	Total volume (µl)
18/130	*TP53* c.916C>T (R306*)	0.48327	1.5205	15	1.85	2.00	1.70	3.70
				10	2.66	2.00	3.32	5.32
				5	5.07	2.00	8.14	10.14
				1	24.40	2.00	46.80	48.80
	NRAS c.34G>T (G12C)	0.45954	1.85863	15	1.60	2.00	1.20	3.20
				10	2.37	2.00	2.74	4.74
				5	4.67	2.00	7.34	9.34
				1	23.05	2.00	44.10	46.10
	PTEN c.795delA (K267fs*9)	1.78544	1.78545	50	1.89	2.00	1.78	3.78
				25	3.68	2.00	5.36	7.36
				10	9.03	2.00	16.06	18.06
				5	17.96	2.00	33.92	35.92
	MAP2K1/MEK1 c.199G>A (D67N)	0.44476	1.75702	15	1.60	2.00	1.20	3.20
				10	2.35	2.00	2.70	4.70
				5	4.57	2.00	7.14	9.14
				1	22.36	2.00	42.72	44.72

Table 2. Example dilutions in the preparation of further standards for TP53 c.916C>T (R306*), NRAS c.34G>T (G12C), PTEN c.795delA (K267fs*9) and MAP2K1/MEK1 c.199G>A (D67N) variants in material 18/130. Dilutions calculated using formula 1.

APPENDIX II. 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/18-130/Additional01.xlsx.