Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

WHO Reference Panel
1st International Reference Panel for genomic KRAS codons 12 and 13 mutations
NIBSC code: 16/250
Instructions for use
(Version 3.0, Dated 16/03/2020)

1. INTENDED USE

The panel comprises eight individually coded ampoules, each containing freeze-dried, purified genomic DNA (gDNA) extracted from human cell lines. The panel comprises seven materials each with a different *KRAS* codons 12 or 13 genotype, a consensus mutation percentage and consensus mutant and total *KRAS* copy numbers per diploid human genome mass. Additionally, the the panel comprises a gDNA homozygous wild-type material for each of the seven *KRAS* codons 12 and 13 genotype (material code 16/266). The materials carrying the *KRAS* mutation of interest may be diluted by application of a calculation specific to each material to produce standards at a range of *KRAS* consensus mutation percentages. The panel is intended for use as primary standards for the calibration of secondary standards, kits, and assays. The materials are not intended as run controls.

The materials were tested by external laboratories and showed suitability as standards in next-generation sequencing (NGS), Sanger sequencing, real-time PCR, pyrosequencing, digital PCR (dPCR), Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis (MassARRAY®), KRAS StripAssay®, high resolution melt analysis (HRM), Amplification Refractory Mutation System-PCR (ARMS-PCR), PCR-Reverse Sequence Specific Oligonucleotide probe technique (PCR-rSSO), minisequencing, and restriction fragment length polymorphism analysis (RFLP).

The panel was established in 2017 by the Expert Committee on Biological Standardization of the World Health Organization (WHO) as the WHO 1st International Reference Panel for genomic *KRAS* codons 12 and 13 mutations, NIBSC code 16/250[1].

These materials should not be put to any other use. Data analysis must be focussed on *KRAS*. No attempt must be made to identify the source material donors.

2. CAUTION

<u>This preparation is not for administration to humans or animals in the human food chain</u>

The cell lines used in the preparation of this panel were tested and found negative for HIV1, HTLV1, HBV, and HCV by PCR. An Epstein Barr virus (EBV)-transformed lymphoblastoid cell line was used in the preparation of the panel. 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 panel was tested in an international collaborative study involving 56 laboratories and 68 testing methods. The genotype and consensus mutation percentage for each of the eight materials was obtained from the most concordant methods, NGS and dPCR (Table 1). End-users are able to further dilute the mutant materials (with wild-type *KRAS* codons 12 and 13 material 16/266, or another wild-type genomic DNA calibrated to material 16/266) using a dilution formula based on the mutant and total *KRAS* copy numbers, to achieve further standards at a range of lower consensus mutation percentages from which assay calibration may be achieved, see section 7 and Appendix I, below.

Material	*KRAS* codon 12 or 13 mutation	Consensus mutation percentage (%)	Consensus mutant *KRAS* copy number per diploid genome mass	Consensus total *KRAS* copy number per diploid genome mass
16/252	p.Gly12Ala	65.7	1.26302	1.92548
16/258	p.Gly12Cys	99.98*	2.86821	2.88025
16/260	p.Gly12Asp	71.5	3.09789	4.34820
16/254	p.Gly12Arg	85.6	14.37815	16.88713
16/256	p.Gly12Ser	99.7	2.16661	2.17463
16/264	p.Gly12Val	49.7	1.27470	2.57349
16/262	p.Gly13Asp	66.9	2.16802	3.25138
16/266	Wild-type	0.0	N/A	2

Table 1. Consensus values for the eight materials of panel 16/250. *KRAS* (NM_033360.3) genotype, consensus mutation percentage, and consensus *KRAS* copy numbers for use in calculating how each mutant material may be diluted to prepare further standards at lower mutation levels, are shown. * Two decimal places to capture low level of wild-type allele presence.

4. CONTENTS

Country of origin of biological material: United Kingdom.

The panel comprises eight individually coded ampoules, each containing approximately 5µg freeze-dried, purified genomic DNA extracted from human cell lines (25µg for material 16/266). The genomic DNAs were 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

<u>No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution</u>

a. Open the ampoule as described in section 6, above.
b. Reconstitute the freeze-dried materials at room temperature with 100µl nuclease-free water, except material 16/266 (wild-type *KRAS*) which should be reconstituted with 200µl nuclease-free water.
c. Transfer each sample to a nuclease-free tube using a pipette, ensuring the maximum available volume is collected.
d. Allow the materials to reconstitute for 1 hour at room temperature and pipette well to mix.
e. Further dilute only material 16/266 (wild-type *KRAS*) with 300µl molecular biology-grade 1x Tris-EDTA buffer (10mM Tris, 1mM EDTA) and pipette well to mix, giving a total volume of 500µl for this material.
f. The DNA concentration for all samples will now be approximately 50ng/µl in 1x Tris-EDTA buffer. Confirm the DNA concentration before use. The

possible appearance of white flecks in the materials should not be of concern.
g. The mutant *KRAS* materials may be combined with material 16/266 (wild-type *KRAS*) to produce standards at any chosen mutation percentage; please see details in Appendix I.
h. Add the required amount to your assay. Materials may be further diluted (with nuclease-free water or suitable buffer) to achieve a DNA concentration appropriate for your assay.
i. 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 1 week 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.who.int/biologicals/expert_committee/BS2317_KRAS_WHO_reference_panel_WHO_BS_2017.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_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 OF THE *KRAS* MUTANT MATERIALS TO GENERATE ADDITIONAL STANDARDS

Each of the mutant *KRAS* codons 12 and 13 materials may be diluted to produce further standards at lower consensus mutation percentages. The preferable diluent is the wild-type *KRAS* codons 12 and 13 material 16/266 included with the panel. However, if insufficient material 16/266 is available to perform the dilutions, an alternative wild-type gDNA may be aligned to material 16/266 and used as the diluent i.e. it should be confirmed as being fully wild-type, diploid, and containing two copies of *KRAS* gene per diplod genome mass.
Notes:
- When preparing the dilutions it is important to calculate the amount of gDNA needed to carry out all the dilution points;
- A minimum of 5 dilution points (including the crude material) is reccomended.
Further details on the dilution response of these materials may be found in the WHO report on the collaborative study to evalutate the proposed WHO 1st International Reference Panel for genomic *KRAS* codons 12 and 13 mutations:
http://www.who.int/biologicals/expert_committee/BS2317_KRAS_WHO_reference_panel_WHO_BS_2017.pdf

Dilutions of the mutant *KRAS* codons 12 and 13 materials may be established as follows:
1. <u>By use of the formula:</u>

((((mutant *KRAS* copy number / wanted %)*100)- total *KRAS* copy number)/2)+1

where the mutant *KRAS* copy number and total *KRAS* copy number can be taken from Table 1, above.

For example, to prepare a standard of consensus mutation percentage 50% for material 16/252 (*KRAS* p.Gly12Ala), the allelic content figures are used thus:

((((1.26302/50)*100)- 1.92548)/2)+1 = 1.30028.

Meaning that a 1 in 1.30 dilution of material 16/252 (1 part material 16/254 plus 0.30 parts wild-type *KRAS* codons 12 and 13 material 16/266) will yield a further standard of consensus mutant percentage 50% *KRAS* p.Gly12Ala, for example, 8.0µl material 16/254, plus 2.4µl material 16/266.

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

2. <u>By reference to dilution curves available from NIBSC:</u>

Use Google Chrome to open the link for an interactive dilution curve for each of the seven mutant *KRAS* codons 12 and 13 materials:
http://www.nibsc.org/documents/ifu/SupplementaryInformation/16-250/InteractiveDilutionCurves.html
For each material, hover the '+' cursor over the dilution curve at the mutation percentage required to see the dilution to be performed.

For example, to prepare a further standard of consensus mutation percentage 50% for material 16/252 (*KRAS* p.Gly12Ala), hover the '+' cursor over 50% on the curve to see the dilution '1.30' i.e. a 1 in 1.30 dilution (1 part material 16/252 plus 0.30 parts wild-type *KRAS* codons 12 and 13 material 16/266) will yield a further standard of consensus mutation percentage 50% *KRAS* p.Gly12Ala, for example 8.0µl material 16/252, plus 2.4µl material 16/266.

Notes:
- The mutant percentage (%) is shown at 5 decimal places to ensure 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. <u>By use of pre-calculated dilutions:</u>

Refer to Table 2, over, for details on the preparation of further standards for each of the materials at a range of consensus mutation percentages.

Material	*KRAS* mutation	Consensus mutant *KRAS* copy number per diploid genome mass	Consensus total *KRAS* copy number per diploid genome mass	Example				
				Wanted *KRAS* mutation %	Dilution to be performed	Volume mutant material (µl)	Volume wild-type material (µl)	Total volume (µl)
16/252	p.Gly12Ala	1.26302	1.92548	50	1.30	8.0	2.40	10.40
				25	2.56	2.0	3.13	5.13
				10	6.35	1.0	5.35	6.35
				5	12.67	1.0	11.67	12.67
16/258	p.Gly12Cys	2.86821	2.88025	50	2.43	2.0	2.86	4.86
				25	5.30	1.0	4.30	5.30
				10	13.90	1.0	12.90	13.90
				5	28.24	1.0	27.24	28.24
16/260	p.Gly12Asp	3.09789	4.34820	50	1.92	5.0	4.62	9.62
				25	5.02	1.0	4.02	5.02
				10	14.32	1.0	13.32	14.32
				5	29.80	1.0	28.80	29.80
16/254	p.Gly12Arg	14.37815	16.88713	50	6.93	1.0	5.93	6.93
				25	21.31	1.0	20.31	21.31
				10	64.45	1.0	63.45	64.45
				5	136.34	1.0	135.3	136.34
16/256	p.Gly12Ser	2.16661	2.17463	50	2.08	2.0	2.16	4.16
				25	4.25	1.0	3.25	4.25
				10	10.75	1.0	9.75	10.75
				5	21.58	1.0	20.58	21.58
16/264	p.Gly12Val	1.27470	2.57349	30	1.84	5.0	4.19	9.19
				20	2.90	2.0	3.80	5.80
				10	6.09	1.0	5.09	6.09
				5	12.46	1.0	11.46	12.46
16/262	p.Gly13Asp	2.16802	3.25138	50	1.54	5.0	2.71	7.71
				25	3.71	1.0	2.71	3.71
				10	10.21	1.0	9.21	10.21
				5	21.05	1.0	20.05	21.05

Table 2. Example dilutions in the preparation of further standards for each of the mutant *KRAS* codons 12 and 13 materials. Dilutions were calculated by use of the formula ((((mutant *KRAS* copy number / wanted %)*100)- total *KRAS* copy number)/2)+1.