Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Reference Reagent
WHO International Reference Reagent for Epidermal growth factor receptor variant L858R (c.2573T>G) genomic DNA
NIBSC code: 20/198
Instructions for use
(Version 2.0, Dated 31/03/2025)

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1. INTENDED USE

The material is intended for calibration of secondary standards, kits, and assays. The material may also be useful for validation and performance monitoring of assays. The material was evaluted in an international collaborative study and shown to be suitable for use in next-generation sequencing (NGS) and digital PCR (dPCR) [1]. Product coded 20/198 was established in 2024 by the World Health Organization (WHO) as the WHO International Reference Reagent for Epidermal growth factor receptor variant p.L858R (c.2573T>G) genomic DNA.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. 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 has associated consensus variant percentage for EGFR c.2573T>G (L858R) of 100% (2 copies per genome equivalent). A consensus variant percentage was obtained form NGS and dPCR data obtained from an international collaborative study. The material may be diluted with NIBSC materials 20/194 and 20/200, to produce a range of EGFR c.2573T>G (L858R) variant percentages.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The coded ampoule contains purified genomic DNA (gDNA) extracted from mutant HT-1080 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. Each ampoule was filled with approximately 500ul of gDNA solution at 10ng/ul DNA (5µg total).

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 20/194 and 20/198 to produce standards at any chosen variant percentage;
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. If further information is required, please contact grmteam@mhra.gov.uk.

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

1. WHO document: https://cdn.who.int/media/docs/default-source/biologicals/call-for-comments/1st-is-for-egfr-genomic-variants-(gdna)_who_bs_2024.2481.pdf?sfvrsn=dc4f36d0_2.

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the significant contributions of all collaborative study participants.
We express our thanks to Simon Patton of EMQN (Manchester, UK) and Klaus Pantel and Claudia Koch of ELBS for connecting us with the participants.
We would also like to extend our gratitude to Paul Matejtschuk, Sara Jane Holmes, James Condron of the Markets, Manufacturing and Logistics (MML) at MHRA. We want to thank the Manufacturing Team for their invaluable work for filling and lyophilisation processes, the Inventory Team for their help on the Accelerated degradation studies and for maintaining material stocks, the Logistics Team for the support of the Collaborative study.
Special thanks to Celso Neto for helping us to set up the dedicated (secure and encrypted) ShareFile Web Page and all the colleagues at MHRA that supported this work. Finally, we would like to extend our heartfelt thanks to all the team members, both present and past, whose dedication and hard work have been instrumental in the success of this project.

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:	No	Irritant:	Unknown
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: 0.002g per ampoule
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No