

**Working Standard
Working Reagent for SARS-CoV-2 RNA
NIBSC code: 20/138
Instructions for use
(Version 1.0, Dated 11/12/2020)**

This material is not for in vitro diagnostic use.

1. INTENDED USE

The Working Reagent for SARS-CoV-2 RNA comprises chimeric lentiviral particles (LVPs) in which the Human Immunodeficiency Virus (HIV-1) genes have been substituted with those of the Wuhan-1 isolate of SARS-CoV-2. The preparation has been evaluated in parallel to the WHO International Standard for SARS-CoV-2 RNA in a WHO International Collaborative study (1). The intended use of the working reagent is as secondary standard or positive control in nucleic acid amplification technique (NAT) based assays for the detection of SARS-CoV-2 RNA.

2. CAUTION

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

This product is a genetically modified material; It is the responsibility of the end user to seek local biosafety approval for the storage and handling of the material in their workplace. 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 Working Reagent 20/138 was calibrated as part of the collaborative study for the establishment of the WHO International Standard for SARS-CoV-2 RNA (NIBSC cat. no. 20/146) and was found to have a unitage of 6.73 Log₁₀ IU/mL, with 95% confidence limits of 6.58 to 6.88 when reconstituted within 0.5mL of molecular grade water or PBS.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial of 20/138 contains 0.5mL of lyophilised, non-infectious, synthetic SARS-CoV-2 RNA packaged within HIV-1 particles. Single nucleotide mutations have been randomly inserted into the SARS-CoV-2 RNA sequences to prevent protein expression and the material was prepared by dividing the SARS-CoV-2 genome into four overlapping fragments [1]. The chimeric LVPs are formulated equimolar in universal buffer comprising 10 mM Tris-HCl (pH 7.4), 0.5% human serum albumin and 1% D-(+)- Trehalose dehydrate and contains a background of 1x10⁵ copies/mL of human genomic DNA. The full consensus sequence of the four chimeric LVPs containing SARS-CoV-2 RNA are deposited under GenBank accessions MW059032, MW059033, MW059034, MW059035

5. STORAGE

The Working Reagent 20/138 should be stored at -20°C or below upon receipt.

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

The material should be reconstituted in 0.5 mL of molecular grade water or PBS. Following addition, the ampoule should be left at ambient temperature for 20 minutes and then mixed thoroughly, avoiding generation of excess foam. Once reconstituted, 20/138 should be diluted in the matrix appropriate to the material/assay being used.

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) Bentley et al., Collaborative Study for the Establishment of a WHO International Standard for SARS-CoV-2 RNA. 2020, WHO Expert Committee on Biological Standardization. WHO/BS/2020.2402

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of the collaborative study participants, particularly in meeting the tight timeframes of this study. We would also like to thank NIBSC Standards Production and Development staff for the formulation and distribution of materials.

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	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2



Other (specify): 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: 0.5 g
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_bi_olefstandardsrev2004.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.