Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Working Standard
NIBSC RR for Lentiviral Vector Integration Copy Number Quantitation
NIBSC code: 19/158
Instructions for use
(Version 3.0, Dated 05/08/2021)

This material is not for in vitro diagnostic use.

1. INTENDED USE

Currently, this candidate standard is not available through the NIBSC website/catalogue but is being made available on request. Users of the material are politely requested to return all raw data relating to use of the standard to NIBSC to support further characterisation of the material. A data reporting file is attached with the IFU. In recognition of this support, up to three sets per lab of the materials will be provided free of charge and users will be asked to cover only the cost of shipping the material.

By requesting the materials, end-users agree for their data to be used by NIBSC for a) internal analysis, b) for deriving a consensus value, c) for the understanding of data discrepancy between ddPCR and qPCR methods and d) for publication. NIBSC will disseminate to end-users the findings and decisions derived from the data.

The candidate standard (18/132) is provided with a diluent (coded 18/142) and with a "theoretical unknown" control sample (coded 18/126). Each ampoule contains freeze-dried, purified genomic DNA extracted from human cells with or without LV DNA integration. The Reference Reagent (18/132) has LV integration in human genome at a nominal value 6.75 log10 LV copies/ampoule (95%CI: 6.70-6.80; k=2). The nominal value was obtained by digital PCR method. The assigned value is nominal, and values derived by end-users may be different from this value. The material is purely for evaluation.

The three materials should be reconstituted in an equal volume of nuclease-free water before assays and the recommended reconstitution volume is 200µL/ampoule. An equal mass (ng) of genomic DNA should be tested in a single assay for the candidate standard and unknown sample(s). End-users can make serial dilutions of 18/132 using 18/142 as the diluent. Sample 18/126 can be used as a theoretical unknown, given a guide value of 6.02 log10 LV copies/ampoule in order to further validate the assay for unknown sample quantitation.

This candidate standard is intended as a run control and for assay validation, but not for clinical quantitation. These materials should not be used for any other purpose. Data analysis must be focussed on lentiviral vector integration.

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 Reference Reagent was tested in an international collaborative study involving thirty-one laboratories from thirteen countries. The consensus lentiviral DNA copies per ampoule was obtained by digital PCR method, the method which gave the highest level of concordance in the collaborative study.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The three individually coded ampoules, each containing approximately 5µg freeze-dried and purified genomic DNA extracted from human cell lines. 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 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 nuclease-free water.
c. Allow the materials to reconstitute for 1 hour at room temperature and then pipette well to mix.
d. Transfer each sample to a nuclease-free tube using a pipette, ensuring the maximum available volume is collected.
e. After being reconstituted in, e.g. 200µL/ampoule, nuclease-free water, the DNA concentration for all samples will now be approximately 25ng/µL based on QuBit quantitation in Tris-EDTA buffer (10mM tris, 1mMEDTA) containing 5mg/mL Trehalose. The possible appearance of white flecks in the materials should not be of concern.
f. Make a series of dilutions of the candidate standard 18/132 in the diluent 18/142 to generate four samples (D to D3) for a standard curve. For example, a 3-fold dilution series has been found to be suitable e.g. D=neat 18/132, D1=1/3 D, D2= 1/3 D1 and D3=1/3 D2.
g. Test equal amount of samples D to D3, diluent 18/142, and, if required, the theoretical unknown 18/126 and unknown samples from your laboratory on the same plate/same assay for a LV sequence. If a qPCR method is to be used, a calculation standard, e.g. a plasmid DNA standard, will need to be included on the same plate and the same assay.
h. Using the estimated and expected LV copies/well for D-D3, a factor may be determined to normalise the LV copies of unknown samples.
Figure 1. Example for how to use the Reference Reagent.

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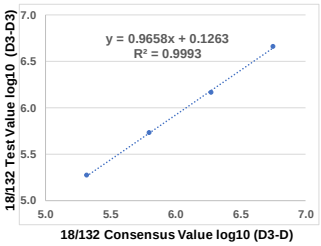
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

	18/132 Consensus Values (log10)	18/132 Test Values (Log10)			
		Test 1	Test 2	Test 3	Mean
18/132 (Log10) D	6.750	6.653	6.610	6.712	6.658
D1	6.273	6.138	6.124	6.226	6.162
D2	5.796	5.707	5.673	5.803	5.728
D3	5.319	5.227	5.239	5.336	5.267



Intercept	Unknown Test value (log10)	Unknown Normalised value (=Unknown test value - Intercept) (log10)
0.126	6.024	5.898 (=6.024-0.1263)

8. STABILITY

Materials are held at NIBSC within assured, temperature-controlled storage facilities. 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 materials 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 one month 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

10. ACKNOWLEDGEMENTS

We would like to thank Professor Didier Trono (EPFL, Lausanne) for his donation of the Lentiviral plasmids to make the project possible. We greatly appreciate the significant contributions of all collaborative study participants.

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 cake		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	Unknown
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	N/A		

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: 0.003g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable**.** **Attached:** No

17. CERTIFICATE OF ANALYSIS

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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.

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