Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
WHO Reference Reagent for the quantitation of Lentiviral Vector copy number by digital PCR
NIBSC code: 18/132d
Instructions for use
(Version 3.0, Dated 10/02/2023)

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1. INTENDED USE

The Reference Reagent is used for the quantitation of Lentiviral Vector copy number by digital PCR (dPCR). The Reference Reagent was established in 2022 by the Expert Committee on Biological Standardization of the World Health Organisation (WHO) and consists of ampoules containing lyophilised, purified genomic DNA for the quantitation of Lentiviral Vector copy number by digital PCR (dPCR) (18/132d) accompanied by ampoules of lypholisied, purified genomic DNA diluent (18/142) for makign further dilutions of the reference reagent 18/132d

The material, coded 18/132d and diluent 18/142 comprises ampoules containing freeze-dried, purified genomic DNA (approximately 5µg) extracted from human cells. The Reference Reagent (18/132d) was tested by external laboratories to demonstrate suitability as a standard for dPCR based quantitation of LV integration. The ampoule coded 18/132d should be reconstituted in nuclease-free water and the recommended reconstitution volume is 200µL/ampoule to give an approximate final DNA concentration of approximately 25ng/µL. Further dilutions of the Reference Reagent (18/132d) should be made in the genomic DNA diluent (18/142) provided with the Reference reagent (approximately 5µg; this ampoule should also be reconstituted in the same volume of 200µL/ampoule nuclease-free water to give the same final DNA concentration of approximately 25ng/µL).

An equal mass (ng) of genomic DNA, e.g. 125ng gDNA/well, for the WHO Reference Reagent and the unknown test sample(s) should be tested in an assay. It is recommended for end-users to make a serial dilution of 18/132d using the genomic DNA diluent (18/142) provided to produce a genomic DNA calculation standard curve for the estimation of LV copy numbers in unknown samples.

These materials should not be put to any other use. 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 (18/132d) and genomic DNA diluent (18/142) were both tested in an international collaborative study involving thirty-one laboratories from thirteen countries and 64 study protocols.

The Reference Reagent (18/132d) unitage assigned is based on data from laboratories performing dPCR methods and is 6.75 log10 (95% CI = 6.71–6.79 log10) LV copies per ampoule and intended for use with dPCR methods.

The genomic DNA diluent (18/142) was confirmed as being free from LV integration and is to be used as a genomic DNA diluent.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The Reference Reagent (Coded 18/132d) and genomic DNA diluent (18/142) are batches of ampoules, each containing approximately 5µg freeze-dried and purified genomic DNA extracted from human cell lines. The genomic DNA 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 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 (recommended volume 200µL/ampoule.

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 then pipette well to mix.

e .If the ampoule has been reconstituted in 200µL water then the DNA concentration 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.

8. STABILITY

Materials are held at NIBSC within assured, temperature-controlled storage facilities. Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference standards. It is the policy of the WHO to not assign an expiry date to either international reference materials. They remain valid with the assigned values and status until withdrawn or amended.

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.

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

World Health Organization

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 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

WHO document WHO/BS/2019.xxx

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

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.