



**WHO International Standard
1st WHO International Standard for Human Papillomavirus (HPV)
Type 16 DNA
NIBSC code: 06/202
Instructions for use
(Version 5.0, Dated 25/11/2019)**

1. INTENDED USE

The 1st International Standard for HPV Type 16 (HPV16) DNA for use in nucleic acid-based assays consists of a freeze-dried preparation of recombinant plasmid pBR322 containing full-length HPV16 DNA cloned via its unique BamH1 site (Quint et al., 2006). The standard has been formulated in a background of purified human genomic DNA, lyophilized in 0.5 ml aliquots and stored at -20 °C. The material was calibrated in an international collaborative study involving 19 laboratories (Wilkinson et al., 2010). The International Standard contains material that is proprietary to third parties and should be used for the sole purpose of calibrating in-house or working standards for the amplification and detection of HPV-16 DNA. The International Standard should not be used for any other purpose and should be discarded after use.

2. CAUTION

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

This material contains DNA derived from C33A cells. 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 1st International Standard for HPV16 DNA (NIBSC code 06/202) has been assigned a unitage of 5×10^6 International Units (IU) per ampoule.

Traceability statement:

It was proposed at a WHO meeting in January 2008 (WHO Meeting Report, 2008) that the instructions for use of the International Standard for HPV16 DNA include the calculations and assumptions used in determining the theoretical HPV16 genome equivalents (GEq) of the bulk material used in formulating the International Standard, thus demonstrating that 1 IU is equivalent to 1 GEq for HPV16 DNA. The definitive unitage of the 1st WHO International Standard for HPV16 DNA therefore remains as IU while the traceability statement would allow users to equate IU with GEq.

Assays for DNA concentration of the recombinant HPV16 plasmid stock preparation were performed in Dr Cosette Wheeler's laboratory, University of New Mexico (UNM). DNA concentrations were determined by absorbance at 260 nm as well as spectrofluorometrically using the Picogreen assay (Invitrogen Corporation, USA). A correlation coefficient of 0.95 or higher was obtained between the two DNA measurements. 10 ng HPV16 plasmid DNA/μl was supplied to NIBSC for formulating the bulk material for subsequent freeze-drying. The UNM laboratory also provided NIBSC with a statement indicating that 1.0×10^{11} GEq/ml for HPV16 is equal to 1.17 ng/μl. 10 ng HPV-16 plasmid DNA/μl plasmid stock preparation is therefore equivalent to 8.547×10^{11} HPV-16 GEq/ml. **NIBSC used this data in formulating the 1st International Standard for HPV Type 16 DNA.**

Formulation of bulk material for the 1st International Standard for HPV Type 16 DNA (NIBSC code 06/202):

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

At NIBSC, the bulk HPV16 plasmid DNA material was prepared according to the formula:

HPV GEq/ml of bulk material = (HPV GEq/ml of plasmid stock x volume plasmid stock) / volume bulk material.

Therefore,

HPV16 GEq/ml of bulk material = $(8.547 \times 10^{11}$ HPV-16 GEq/ml plasmid stock) x (0.02223 ml HPV-16 plasmid stock) / 1900 ml HPV-16 bulk material = 1.0×10^7 HPV-16 GEq/ml bulk material
The HPV-16 DNA bulk material was subsequently freeze-dried in 0.5 ml aliquots.

Certain assumptions are required for equating IU to GEq for the 1st International Standard for HPV16 DNA: 1) 1.0×10^{11} GEq/ml for HPV16 is equal to 1.17 ng/μl. 2) There is no loss in activity of the HPV16 DNA upon lyophilization. 3) The recombinant HPV-16 plasmid DNA accurately mimics the activity of HPV16 viral DNA in biological samples.

Independent calculation of GEq/ml for recombinant HPV-16 plasmid DNA.

NIBSC also independently calculated the genome equivalence of the HPV-16 plasmid stock preparation and bulk preparation in which the molecular weights of the full-length HPV16 genome and pBR322 DNA were based on sequence content using BioEdit Sequence Alignment Editor v7.0.5.3 (Tom Hall, Isis Pharmaceuticals Inc., USA). The sequences used for determining the molecular weights are GenBank Accession number J01749.1 for pBR322 and the reference sequence for HPV16 (Accession K02718).

BioEdit data

DNA molecule: HPV16 Accession K02718
Length = 7904 base pairs
MW= 4786756.00 Daltons, double stranded

DNA molecule: cloning vector pBR322

Length = 4361 base pairs
MW= 2653867.00 Daltons, double stranded

Formulae

GEq/ml of the HPV plasmid stock was calculated according to the formula:
GEq/ml of the HPV plasmid stock = (DNA concentration of HPV plasmid stock) x (MW of HPV DNA + MW of pBR322)⁻¹ x (Avogadro's Number)
where Avogadro's Number = 6.022×10^{23} molecules/mol
GEq/ml of the bulk HPV DNA materials was calculated according to the formula:
HPV GEq/ml of bulk material = (HPV GEq/ml of plasmid stock x volume plasmid stock) / volume bulk material.

Calculation

The recombinant HPV16 plasmid stock preparation was supplied to NIBSC at a concentration of 10 ng/μl. Using the MW determinations shown above, the GEq/ml of the HPV16 plasmid stock is:
= $(10 \times 10^{-9} \text{ g/}\mu\text{l}) \times (\text{mol}/(7440623 \text{ g}) \times (6.022 \times 10^{23} \text{ molecules/mol}))$
= 8.093×10^9 molecules/μl
= 8.093×10^{11} molecules/ml
= 8.093×10^{11} HPV16 GEq/ml
22.23μl of the recombinant HPV16 plasmid stock was diluted to a final volume of 1900ml, therefore,
HPV-16 GEq/ml of bulk material = $(8.093 \times 10^{11}$ HPV16 GEq/ml plasmid stock) x (0.02223 ml HPV16 plasmid stock) / 1900 ml HPV16 bulk material = 0.947×10^7 HPV16 GEq/ml bulk material

4. CONTENTS

Country of origin of biological material: United Kingdom.



Each ampoule contains the lyophilized equivalent of 0.5 ml HPV16 plasmid DNA in 10mM Tris buffer pH7.4 containing 1mM EDTA, 5 mg/ml trehalose and $\sim 1 \times 10^6$ human GEq/ml derived from C33a cells.

5. STORAGE

The ampoule should be stored at -20 °C or below on 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 1st International Standard for HPV16 DNA contains high copy number template. There is a high risk of HPV16 plasmid DNA contamination via aerosolization upon opening of the glass ampoule. The material must be opened and handled in a separate laboratory environment, away from other pre-amplification components such as reagents, labware and samples.

The material is supplied lyophilized and, before use, should be reconstituted in 0.5 ml sterile nuclease-free water. Ensure that the inside surface of the ampoule is wetted with the added water so that any particles of freeze-dried material adhering to the glass are reconstituted. The reconstituted material has a final concentration of 1×10^7 IU/mL.

The reconstituted material is suitable for calibration of in-house or working standards for the amplification and detection of HPV16 DNA. The material is not suitable for calibrating or assessing extraction, precipitation or centrifugation procedures. NIBSC can provide guidance for the use of the International Standard for HPV16 DNA in assays where the extraction step cannot be separated from the amplification step (e.g. sample-in, answer-out platforms). The material has NOT been calibrated for human DNA nucleic acid amplification techniques.

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.

Degradation studies on 06/202 and 06/206 indicate that the freeze-dried material is extremely stable and suitable for long-term storage. (Wilkinson et al., 2010). Users should determine the stability of the reconstituted material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

Ferguson, M., DE. Wilkinson, and T. Zhou. 2009. WHO meeting on the standardization of HPV assays and the role of the WHO HPV Laboratory Network in supporting vaccine introduction held on 24-25 January 2008, Geneva, Switzerland. *Vaccine* 27:337-347.

Quint, WG., SR. Pagliusi, N. Lelie, E-M. de Villiers, CM. Wheeler, and the World Health Organization Human Papillomavirus DNA. International Collaborative Study Group. 2006. Results of the First World Health Organization International Collaborative Study of Detection of Human Papillomavirus DNA. *J. Clin. Microbiol.* 44:571-579.

Wilkinson, DE., SA. Baylis, D. Padley, AB. Heath, M. Ferguson, SR. Pagliusi, WG. Quint, and CM. Wheeler. 2010. Establishment of the 1st World Health Organization international standards for human papillomavirus type 16 DNA and type 18 DNA. *Int J Cancer* 126:2969-2983.

Human Papillomavirus Laboratory Manual. First edition, 2009. WHO/IVB/10.12
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Eklund C, Forslund O, Wallin KL, Dillner J. Continuing global improvement in human papillomavirus DNA genotyping services: The 2013 and 2014 HPV LabNet international proficiency studies. *J Clin Virol.* 2018;101:74-85.

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

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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: Lyophilized powder | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: No | Irritant: No |
| Flammable: No | Handling: See caution, Section 2 |
| Other (specify): | |
| 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 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_biologicalstandardsrev2004.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.