



**WHO International Standard  
1st WHO International Standard for Human Papillomavirus (HPV)  
Type 18 DNA  
NIBSC code: 06/206  
Instructions for use  
(Version 4.0, Dated 25/11/2019)**

**1. INTENDED USE**

The 1st International Standard for HPV Type 18 (HPV18) DNA for use in nucleic acid-based assays consists of a freeze-dried preparation of recombinant plasmid pBR322 containing full-length HPV18 DNA cloned via its unique EcoR1 site (Quint et al., 2006). The EcoR1 cloning site is located within the E1 gene. 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 HPV18 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.**

The 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 HPV18 DNA (NIBSC code 06/206) has been assigned a unitage of  $5 \times 10^6$  International Units (IU) per ampoule.

**Traceability statement:**

It was proposed at a WHO meeting in January 2008 (Ferguson et al., 2009) that the instructions for use of the International Standard for HPV18 DNA include the calculations and assumptions used in determining the theoretical HPV18 genome equivalents (GEq) of the bulk material used in formulating the International Standard, thus demonstrating that 1 IU is equivalent to 1 GEq for HPV18 DNA. The definitive unitage of the 1st WHO International Standard for HPV18 DNA therefore remains as IU while the traceability statement would allow users to equate IU with GEq. Assays for DNA concentration of the recombinant HPV18 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. Prior to delivery to NIBSC, the HPV18 plasmid stock preparation was adjusted to a final concentration of 5 ng HPV18 plasmid DNA/ $\mu$ l. The UNM laboratory also provided NIBSC with a statement indicating that  $1.0 \times 10^{11}$  GEq/ml for HPV18 is equal to 1.27 ng/ $\mu$ l. Five ng HPV18 plasmid DNA/ $\mu$ l plasmid stock preparation is therefore equivalent to  $3.937 \times 10^{11}$  HPV18 GEq/ml. **NIBSC used this data in formulating the 1st International Standard for HPV Type 18 DNA.**

**Formulation of bulk material for the 1st International Standard for HPV Type 18 DNA (NIBSC code 06/206):**

At NIBSC, the bulk HPV18 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,

HPV18 GEq/ml of bulk material =  $(3.937 \times 10^{11}$  HPV18 GEq/ml plasmid stock) x (0.04826 ml HPV18 plasmid stock) / 1900 ml HPV18 bulk material =  $1.0 \times 10^7$  HPV18 GEq/ml bulk material  
The HPV18 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 HPV18 DNA: 1)  $1.0 \times 10^{11}$  GEq/ml for HPV18 is equal to 1.27 ng/ $\mu$ l; 2) there is no loss in activity of the HPV18 DNA upon lyophilization; 3) the recombinant HPV18 plasmid DNA accurately mimics the activity of HPV18 viral DNA in biological samples.

**Independent calculation of GEq/ml for recombinant HPV18 plasmid DNA.**

NIBSC also independently calculated the genome equivalence of the HPV18 plasmid stock preparation and bulk preparation in which the molecular weights of the full-length HPV18 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 HPV18 (Accession X05015).

**BioEdit data**

DNA molecule: HPV18 Accession X05015  
Length = 7857 base pairs  
MW= 4763530.00 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 stocks 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)<sup>-1</sup> x (Avogadro's Number)  
where Avogadro's Number =  $6.022 \times 10^{23}$  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 HPV18 plasmid stock preparation was supplied to NIBSC at a concentration of 5 ng/ $\mu$ l, therefore the GEq/ml of the HPV18 plasmid stock is:  
=  $(5 \times 10^{-9}$  g/ $\mu$ l) x (mol/(7417397 g) x ( $6.022 \times 10^{23}$  molecules/mol))  
=  $4.059 \times 10^6$  molecules/ $\mu$ l  
=  $4.0591 \times 10^{11}$  molecules/ml  
=  $4.059 \times 10^{11}$  HPV18 GEq/ml  
48.26 $\mu$ l of the recombinant HPV18 plasmid stock was diluted to a final volume of 1900ml, therefore:  
HPV18 GEq/ml of bulk material =  $(4.059 \times 10^{11}$  HPV18 GEq/ml plasmid stock) x (0.04826 ml HPV18 plasmid stock) / 1900 ml HPV18 bulk material =  $1.031 \times 10^7$  HPV18 GEq/ml bulk material

**4. CONTENTS**

Country of origin of biological material: United Kingdom.  
Each ampoule contains the lyophilized equivalent of 0.5 ml HPV18 plasmid DNA diluted 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 HPV18 DNA contains high copy number template. There is a high risk of HPV18 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 X 10<sup>7</sup> IU/mL. The reconstituted material is suitable for calibration of in-house or working standards for the amplification and detection of HPV18 DNA (WHO/IVB/10.12). The material should NOT be used to calibrate or assess extraction, precipitation or centrifugation procedures. NIBSC can provide guidance for the use of 06/206 in assays where the extraction step cannot be separated from the amplification step (e.g. sample-in, answer-out platforms). This 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  
[http://www.who.int/immunization/documents/WHO\\_IVB\\_10.12/en/index.html](http://www.who.int/immunization/documents/WHO_IVB_10.12/en/index.html).

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](mailto: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](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](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](mailto: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: 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](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](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.