



**WHO Reference Reagent  
Factor V Leiden, Human gDNA. 1st International Genetic  
Reference Panel  
NIBSC code: 04/224  
Instructions for use  
(Version 5.0, Dated 06/12/2017)**

### 1. INTENDED USE

The ampoules contain freeze-dried purified gDNA samples extracted from EBV transformed cell lines. They are intended for use as a reference panel in genetic tests for Factor V Leiden (FVL). The panel was established in 2004 as the 1<sup>st</sup> International Genetic Reference Panel for Factor V Leiden by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO). N.B. these materials should not be put to any other use.

### 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

There is no unitage assigned to these materials.

### 4. CONTENTS

Country of origin of biological material: United Kingdom.

The panel comprises three individually coded ampoules, each containing approximately 10 µg of human gDNA;

03/254 Wild Type Factor V

03/248 Factor V Leiden Heterozygote

03/260 Factor V Leiden Homozygote

The DNA samples were extracted using a 'salting out' method and suspended in Tris/EDTA buffer with 5 mg/ml Trehalose as an excipient before freeze-drying.

The factor V gene in all three preparations has been sequenced between 378bp 5' and 322bp 3' of the FVL SNP. Only the expected G>A polymorphism in exon 10 was detected. The remaining sequence in all three preparations was identical to the published sequence Ensemble Gene ID ENSG0000056213.

### 5. STORAGE

Store all unopened ampoules of the freeze-dried preparations 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

a. Open ampoules as described in section 6. above.

b. Reconstitute freeze-dried material at room temperature with 100µL of sterile nuclease-free water.

c. Transfer the entire contents to nuclease-free tubes.

d. If your normal pipette tips do not reach the samples at the bottom of the ampoule, please use long tips provided.

e. We recommend that the material is used directly after reconstitution and is not stored beyond this point, but if this is desired, then the material should be stored in sealed tubes between +2 to +8°C if the samples are to be tested within 3 months. For longer periods, store in aliquots at -20°C or below. Care should be taken to avoid cross-contamination with other samples.

### 8. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. Reference materials are held at NIBSC within assured, temperature controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

### 9. REFERENCES

The following publication describes the International Collaborative Study which was carried out in order to characterise the panel; E Gray, JR Hawkins, M Morrison, M Hawkins, E Byrne, S Kitchen, I Jennings, M Makris, FE Preston, and P Metcalfe. Establishment of the 1st International Genetic Reference Panel for Factor V Leiden (G1691A), Human gDNA. Thrombosis and Haemostasis 2006, 96 215-219.

### 10. ACKNOWLEDGEMENTS

We would like thank the staff of the UK NEQAS for Blood Coagulation and the Royal Hallamshire Hospital, Sheffield, UK for supplying blood samples.

### 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

based on the report of the international collaborative study which established their suitability for the intended use.

Physical and Chemical properties	
Physical appearance: freeze-dried solid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains 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](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

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 0.0028 - 0.0030g
<b>Toxicity Statement:</b> Toxicity not assessed
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> 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\\_biodefstandardsrev2004.pdf](http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biodefstandardsrev2004.pdf) (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS)