



**WHO International Standard
Prader Willi & Angelman Syndromes, Human gDNA, 1st
International Genetic Reference Panel
NIBSC code: 09/140
Instructions for use
(Version 6.0, Dated 06/11/2015)**

1. INTENDED USE

The ampoules contain freeze-dried purified genomic DNA (gDNA) extracted from Epstein Barr virus (EBV)-transformed cell lines. They are intended for use as a reference panel in genetic testing for Prader Willi & Angelman syndromes. Data analysis must be focussed on Prader Willi & Angelman syndrome-relevant loci. This panel was established in 2009 by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO) as the 1st WHO International Genetic Reference Panel for Prader Willi & Angelman Syndromes, NIBSC code 09/140¹.
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.

Each material was tested and found to be negative for HIV1, HTLV1, HBV and HCV by PCR. 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 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 panel comprises 6 individually coded ampoules, each containing approximately 5 µg human gDNA;
07/230 Angelman syndrome, maternal deletion (class I deletion)
07/232 Angelman syndrome, UBE3A point mutation
07/234 Angelman syndrome, paternal uniparental disomy or imprinting centre defect
07/236 Prader Willi syndrome, paternal deletion (unbalanced chromosome 15;19 translocation)
07/238 Prader Willi syndrome, maternal uniparental disomy
07/240 Prader Willi syndrome, paternal deletion (class I deletion).
The panel was tested in an international collaborative study involving 37 laboratories and the genotypes confirmed by the use of methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), methylation-specific PCR (MS-PCR), UBE3A sequence analysis, Southern blotting, microsatellite analysis, methylation-sensitive PCR, DHPLC and methylation-specific melting analysis. UBE3A sequence analysis of material 07/232 confirmed the mutation as c.1234A>T;p.Lys412Stop (UBE3A nomenclature following HGVS guidelines using GenBank accession number U84404.1 and numbering from the "A" of the "ATG" start codon as nucleotide 1 in accordance with HGVS guidelines).
Normal variation in methylation levels and copy number changes in the Prader Willi & Angelman syndromes critical region have been reported in healthy individuals² and may be detected in MS-MLPA; atypical signals for some of the SNRPN and NDN probes were reported for the materials in the collaborative study (although the overall interpretations were not affected).
In MS-PCR, the appearance of a very faint second band in samples 07/230, 07/234, 07/236, 07/238 & 07/240 may not necessarily be due

to sub-optimal assay conditions, but rather due to a cell culture methylation artefact or low level methylation mosaicism in these materials.

Overall results for MS-MLPA and MS-PCR are as follows³:

	MS-MLPA			MS-PCR	
	Deletion	Maternal methylation pattern	Paternal methylation pattern	Maternal allele	Paternal allele
07/230	✓	✗	✓	✗	✓
07/232	✗	✓	✓	✓	✓
07/234	✗	✗	✓	✗	✓
07/236	✓	✓	✗	✓	✗
07/238	✗	✓	✗	✓	✗
07/240	✓	✓	✗	✓	✗

✓ and ✗ indicate the expected result from each of the methods.

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

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

- Open ampoule as described in section 6. above.
- Reconstitute freeze-dried material at room temperature with 100 µl sterile nuclease-free water.
- Transfer the entire contents to a nuclease-free tube.
- Allow the material to stand for 1 hour at room temperature and pipette well to mix before use.
- Measure the DNA concentration before use and add the required amount to your assay.
- We recommend that the material is used on the day it is reconstituted and is not stored. However, in our hands the reconstituted freeze-dried human genomic DNA materials were stable for up to 3 months at +4°C (as demonstrated by agarose gel electrophoresis) or for longer periods at -20°C or below. Care should be taken to avoid cross-contamination with other samples.

8. STABILITY

NIBSC follows the policy of the WHO with respect to its reference materials. It is the policy of the WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Accelerated degradation studies indicate that the freeze-dried materials in ampoules are stable after storage at +56°C and +45°C for at least 3 years. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

- ¹WHO document WHO/BS/09.2105
- ²Ramsden SC et al. Practice guidelines for the molecular analysis of Prader-Willi and Angelman syndromes. BMC Medical Genetics 2010, 11:70

³Boyle J et al. Establishment of the first WHO international genetic reference panel for Prader Willi and Angelman syndromes. Eur J Hum Genet. 2011 Aug;19(8):857-64

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

NIBSC Genomic Reference Materials:

http://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials.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: 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 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: 0.003 g per ampoule

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.