



**WHO International Standard
1st International Standard for human Proinsulin
NIBSC code: 09/296
Instructions for use
(Version 1.0, Dated 17/12/2014)**

1. INTENDED USE

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2010) the need for a replacement for the International Reference Reagent (IRR) for human proinsulin, coded 84/611. A preparation of proinsulin, coded 09/296, was ampouled and evaluated for its suitability to serve as a WHO International Standard by international collaborative study. It was established as the 1st International Standard for human Proinsulin by the WHO ECBS in October 2014. This replaces the International Reference Reagent, coded 84/611, as the primary reference material for the calibration of immunoassays of Proinsulin.

2. CAUTION

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

The material is not of human or bovine origin. 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

7.0 µg per ampoule with an expanded uncertainty (95% confidence; $k=2.36$) of 6.4-7.7.

4. CONTENTS

Country of origin of biological material: USA.

Each ampoule contains the residue, after freeze-drying, of 0.5 ml of a solution which contained:

7.0 µg Proinsulin
0.41 mg di-Sodium hydrogen phosphate anhydrous
0.29 mg Sodium di-hydrogen phosphate monohydrate
2.5 mg Trehalose

5. STORAGE

Unopened ampoules should be stored at -20°C

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

For practical purposes each ampoule contains the same quantity of proinsulin. The entire content of each ampoule should be completely dissolved in an accurate volume of suitable diluent. If extensive dilutions are prepared, a carrier protein (0.05 - 0.1% w/v BSA or HSA) should be added. The ampoules do not contain bacteriostat and a solution of the reagent should not be assumed to be sterile.

COLLABORATIVE STUDY

An international collaborative study, carried out in 9 countries, by 17 laboratories, was conducted in three phases to assign a mass content to 09/296.

Phase 1 involved the assignment of a value to a primary calibrant in mass units by amino acid analysis and UV spectroscopy.

Phase 2 applied this value to the calibration of 09/296 by reverse phase-HPLC assay.

Phase 3 of the study compared the immunological activity of 09/296 with the existing standard, 84/611, by current immunoassays to assess its suitability to serve as an International Standard.

Estimates from the HPLC calibration indicated the content of 09/296 to be 7.0 µg per ampoule. Results of Phase 3 indicate that 09/296 shows appropriate immunological activity, and appears sufficiently stable on the basis of a thermally accelerated degradation study, and is thus suitable to serve as an International Standard for immunoassays of human proinsulin.

8. STABILITY

Stability based on HPLC analysis of thermally accelerated degradation samples showed a predicted yearly loss of activity when stored at -20°C of 0.013% and a predicted yearly loss of proinsulin content of 3.7% at 20°C. These results indicate that 09/296 is likely to be highly stable under long term storage conditions at -20°C and that the material will also be stable during normal shipping at ambient temperatures.

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

In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

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

9. REFERENCES

10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr B. Frank and Eli Lilly & Co., Indianapolis, USA for providing the bulk material and to the participants of the collaborative study.

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 freeze dried powder | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: Yes | 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 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: 3mg |
| 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_biol_efstandardsrev2004.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.