



**WHO International Standard
Insulin, Human
NIBSC code: 83/500
Instructions for use
(Version 4.0, Dated 23/09/2010)**

1. INTENDED USE

Establishment of the International Standard for human Insulin was authorized at the 37th Meeting of the WHO Expert Committee of Biological Standardization. This material replaces the 4th IS of bovine/porcine insulin as the standard for the bioassay of human insulin. The 4th IS is discontinued.

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

ONE INTERNATIONAL UNIT of human insulin is the activity contained in 0.03846mg of the international standard for human insulin, by definition.

4. CONTENTS

Each ampoule contains approximately 50mg of hydrated crystals of human insulin, together with nitrogen gas at atmospheric pressure.

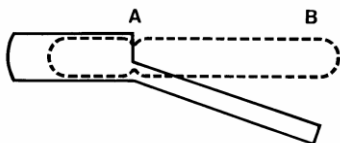
5. STORAGE

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

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

The content of the ampoule is variable. Aliquots of insulin crystals should be accurately weighed after allowing the ampoule to equilibrate at room temperature in a desiccator. Unused portions of the ampoule should be stored desiccated at -20°C or below

8. PREPARATION OF AMPOULES

8.1 Bulk Material

Approximately 200g of human insulin crystals prepared by enzymic modification of porcine insulin were generously donated by Novo Industri A/S, Copenhagen. The material contained approximately 98% insulin, 1% desamido insulin and 1% other insulin-related impurities, when examined by either reverse-phase HPLC or electrophoresis. Less than 0.3% of insulin aggregates were detected by gel-filtration.

8.2 Distribution into ampoules

The batch of ampoules coded 83/500 was prepared by distributing approximately 50mg aliquots of hydrated insulin crystals into glass ampoules under conditions of controlled humidity. Ampoules were purged with nitrogen, sealed by heat-fusion of the glass, and have since been stored at -20°C in the dark.

9. COLLABORATIVE STUDY

The preparation in ampoules coded 83/500 was evaluated by an international collaborative study in which twenty-three laboratories in sixteen countries participated. The study was organized with the following aims:- to assess the suitability of preparations of highly purified bovine, porcine and human insulins to serve as standards for the replacement of the International Standard for Insulin, to relate each of these preparations to the 4th International Standard for Insulin by bioassays and to characterize these preparations by physical, biochemical and immunological procedures used for the analysis of insulin. Information was also sought regarding any effects the species of origin and purity of the insulin might have on results obtained with bioassay procedures currently in use. The study also sought, if possible, to compare human insulin obtained by recombinant DNA technology, by modification of porcine insulin, and from human pancreases.

9.1 Estimate of bioactivity

Bioassay data using the mouse blood-glucose, rabbit blood-glucose and mouse convulsion methods were contributed to the study. The combined estimate for all methods was 26.0 IU/mg (95% limits 25.5-26.4).

9.2 Assigned unitage

The assigned potency is 0.03846mg in one International Unit of Insulin, human (26.0 IU/mg). The material is intended to serve as a standard for the assay of human insulin preparations, either by biological methods or by physicochemical techniques such as HPLC. Porcine and bovine insulin preparations should be assayed against the corresponding international standards (porcine, code 83/515; bovine, code 83/511).

10. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. The lower 95% limit for the predicted loss of activity of the material stored at -20°C, based upon assays of thermally degraded samples, was less than 0.1% per year.

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. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Unopened ampoules 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.

11. REFERENCES

Bristow, A.F., Gaines Das, R.,E. and Bangham, D.R. (1988). WHO international standards for highly purified human, porcine and bovine insulins. J.Biol.Stand. 16: 165-178.



12. ACKNOWLEDGEMENTS

Acknowledgements are due to Novo Industri A/S, Copenhagen, Denmark, who generously donated, through the good offices of Dr. J. Schlichtkrull, the insulin; to the Standards Processing Division of NIBSC for ampouling; and to the participants in the collaborative study.

13. 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

14. 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

15. 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.

16. 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 powder	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.	

17. 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.

18. 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: 50mg
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.