



**International Ref. Reagent
IRR Proinsulin, Human, for Immunoassay
NIBSC code: 84/611
Instructions for use
(Version 4.0, Dated 28/03/2013)**

1. INTENDED USE

At its 37th meeting, the WHO Expert Committee on Biological Standardization authorized the establishment of the preparation coded 84/611 as the International Reference Reagent for Proinsulin. The Committee, however, noted that the data from the collaborative study were limited. Accordingly, 84/611 is assigned a nominal ampoule content of 6.0µg/ampoule

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 probably will 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

6.0µg per ampoule

The µg value of 84/611 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 84/611 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.05%.

4. CONTENTS

Each ampoule contains the freeze-dried residue of a solution which contained in 1ml:-

Purified human proinsulin	approx 6µg
Peptidase-free human serum albumin	1mg
Lactose	5mg

Nitrogen gas at slightly less than atmospheric pressure

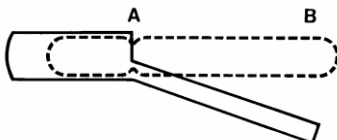
5. STORAGE

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

For practical purposes each ampoule contains the same quantity of the above materials. The entire contents of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. No attempt should be made to weigh out portions of the freeze-dried powder.

For economy in use, it is recommended that the solution, without further dilution, is sub-divided into several small containers and stored at -30°C or below. To maintain full activity of such stored aliquots, it is suggested that the solution of ampoule contents and its subdivision and freezing by using CO₂/ethanol or liquid N₂ is done rapidly. A dilute solution prepared for use in an assay should be kept cool (e.g. 4°C) and should contain not less than 0.1% w/v carrier protein (free of proteolytic enzymes). Repeated freezing and thawing should be avoided. The material has not been sterilized and the ampoules contain no bacteriostat.

8. PREPARATION OF AMPOULES

Bulk Material

Human proinsulin (25mg) made by r-DNA technology in bacteria was generously donated to WHO by Eli Lilly and Co, Indianapolis, USA, through the good offices of Dr. B. Frank. The material was 97% human proinsulin by HPLC, with other peaks being pro-insulin related (eg. desamido proinsulin). The amino-acid analysis of the material was consistent with the sequence of human proinsulin.

Distribution into ampoules

Ampoules coded 84/611 containing a nominal 10g lyophilized proinsulin, human serum albumin and lactose were prepared according to the methods recommended for international biological standards. A weighed portion of the proinsulin was dissolved in a sterile solution containing 0.5% lactose and 0.1% peptidase-free human serum albumin. This solution was passed through a filter (mean pore diameter 0.45µm) and distributed in 1.0ml aliquots into ampoules. Filled solutions were lyophilized, and after secondary desiccation, were sealed under nitrogen by heat fusion of the glass and stored at -20°C in the dark.

9. COLLABORATIVE STUDY

Three laboratories in Denmark evaluated the material coded 84/611 with the following aims: (1) to estimate the human proinsulin content of the ampouled preparation and to assign a defined content to each ampoule; (2) to assess the stability of the preparation to serve as a standard for immunoassays of human proinsulin; (3) to assess the suitability of the ampouled preparation of proinsulin by comparison with ampoules that had been subjected to accelerated thermal degradation.

Estimate of immunoactivity

Two laboratories calibrated the material in terms of independent local standards. Both estimated the content to be 6µg per ampoule.

10. STABILITY

84/611 did not exhibit any loss of immunoactivity upon accelerated thermal degradation.

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.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. 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. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.



11. REFERENCES

Bristow, A.F. & Gaines-Das, R.E. (1988). WHO international reference reagents for human proinsulin and human C-peptide. J. Biol. Stand. 16: 179-186.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr B. Frank and Eli Lilly & Co. for providing the bulk material and 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. 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.

15. 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.	

16. 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.

17. 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: 6mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No