Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

International Ref. Reagent
IRR Proinsulin, Porcine, for Immunoassay
NIBSC code: 84/528
Instructions for use
(Version 3.0, Dated 12/12/2007)

1. INTENDED USE

Establishment of the International Reference Reagent for Porcine Proinsulin was authorised at the 37th meeting of the WHO Expert Committee on Biological Standardisation.

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2. CAUTION

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

The preparation contains an excipient of human origin which has been tested and found negative for HBsAg, and HIV antibody. The preparation has subsequently been tested and found negative for HCV RNA 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 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

20 micrograms per ampoule, by definition

4. CONTENTS

Each ampoule contains the freeze-dried residue of 1.0ml of a solution which contained in 1ml:-

Purified porcine proinsulin	25µg (nominal)
Lactose	5mg
Human serum albumin	1mg

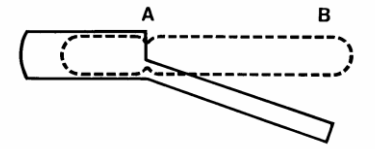
Nitrogen gas at slightly less than atmospheric pressure

5. STORAGE

Unopened ampoules should be stoted 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 weight 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 stores 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 CO_2 /ethanol or liquid N_2 is done rapidly. A dilute solution prepared for use in an assay should be kept cool (eg 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
Approximately 50mg porcine proinsulin, purified from porcine pancreas, was generously donated to WHO by Novo Industri A/S, Denmark. The preparation was homogenous by reversed-phased HPLC on C18 silica (> 98% pure) and the amino acid composition was in agreement with the published values for the structure of porcine proinsulin.
Distribution into ampoules
The batch of ampoules coded 84/528 was prepared according to the procedures used for international biological standards (29th ECBS Report, 1978). A weighed portion of the proinsulin was dissolved in a sterile solution containing 0.1 w/v peptidase-free human serum albumin and 0.5% w/v lactose. This solution was passed through a membrane filter (mean pore diameter 0.4m) and distributed in 1.0ml aliquots into ampoules. The mean weight of the filled aliquots was 1.00218g, with a maximum range of 0.6%. The ampouled solution was lyophilized, and after secondary desiccation, the ampoules, containing pure dry nitrogen, were 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 84/528 was evaluated by international collaborative study in which six laboratories in five countries took part. The study was organized (1) to calibrate 84/528 in terms of local standards, (2) to assess the stability of 84/528, and (3) to assess the suitability of 84/528 to serve as a standard for the assay of proinsulin in insulin formulations.
Estimate of immunoactivity
The mean of all estimates of proinsulin content, in terms of local standards, was approximately 20 µg per ampoule.

10. STABILITY

84/528 did not exhibit any loss of immunoactivity after storage for 2 months at elevated temperatures.
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. 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. REFERENCE

Gaines-Das, R.E. & Bristow, A.F. (1988). WHO international reference reagents for bovine and porcine proinsulins. J.Biol. Stand. 16:187-193.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Novo Industri A/S, Copenhagen, Denmark, for providing the material; the Standards Processing Division of NIBSC for ampouling; and the participants in the collaborative study.

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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

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