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.

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 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 by 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.

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 material. 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 CO2/ethanol or liquid N2 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 0.4μm) 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

12. ACKNOWLEDGEMENTS
Grateful acknowledgements are due to Novo Industri A/S, Copenhagen, Denmark, for providing the material; the Standards Processing Division of NIBSC for ampling; 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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze dried powder</td>
</tr>
<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains material of human origin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Action on Spillage and Method of Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

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