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.

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
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.

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 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 internati
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 ampouling; 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/jc10m/
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>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze dried powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material of human origin</td>
</tr>
</tbody>
</table>

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

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

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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

Page 2 of 2