International Ref. Reagent

C-Peptide of Human Insulin, International Reference Reagent

NIBSC code: 84/510

Instructions for use

(Version 5.0, Dated 28/03/2013)

1. INTENDED USE

Establishment of the International Reference Reagent for Insulin C-peptide was authorised at the 37th Meeting of the WHO Expert Committee on Biological Standardization.

2. CAUTION

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

The material is not of human or bovine origin.

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

10 µg per ampoule, by definition.

The µg value of 84/510 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 84/510 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.08%.

4. CONTENTS

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

- Purified human insulin C-peptide 10 µg (nominal)
- Peptidase-free bovine 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.

![Diagram of ampoule opening device](image)

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 (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

Human insulin C-peptide made by rDNA procedures in E. coli, was generously donated to WHO by Eli Lilly & Co., Indianapolis, USA, through the good offices of Dr. B. Frank. The material was essentially homogenous by HPLC, and amino acid analysis was consistent with the published sequence of residues 33-63 of human proinsulin.

Distribution into ampoules

Ampoules coded 84/510 were prepared according to the procedures used for international biological standards. A weighed portion of C-peptide was dissolved in a sterile solution containing 0.1 w/v peptidase-free bovine serum albumin and 0.5% w/v lactose. This solution was passed through a membrane filter (mean pore diameter 0.45m) and distributed in 1.0ml aliquots into ampoules. The mean weight of the filled aliquots was 1.00218g, with a maximum range of 0.06%. 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 -70°C in the dark.

9. COLLABORATIVE STUDY

The preparation in ampoules coded 84/510 was evaluated by international collaborative study in which 5 laboratories in 4 countries took part. The aims of the study were:

1) To estimate the C-peptide content of the ampouled preparation 84/510 in terms of the participants’ local standards and to assign a defined content to each ampoule.

2) To assess the suitability of the preparation to serve as a standard for the immunoassay of C-peptide by comparison of displacement curves of the material with those of participants’ local standards.

3) To assess the suitability of the preparation to serve as a standard for the immunoassay of clinical serum specimens by comparisons with sera from patients with various disorders including insulinoma, diabetes and abnormal oral glucose tolerance tests.

4) To assess the stability of the preparation of C-peptide by comparison with ampoules that had been subjected to accelerated thermal degradation.

Estimates of immunoactivity

The weighted geometric mean of all assays, in terms of local standards, was 9.95 micrograms per ampoule (95% confidence limits 9.07-10.88).

Assigned unitage

The assigned unitage of 10 micrograms per ampoule is consistent with the nominal content.

10. STABILITY

No loss of immunoactivity was observed upon storage at up to 45°C for 2-3 months.

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

12. ACKNOWLEDGEMENTS
Grateful acknowledgements are due to Dr. B. Frank, Eli Lilly & Co, Indianapolis, Indiana, USA, for providing the material; 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. 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: solid</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): None</td>
<td></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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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.</td>
</tr>
<tr>
<td>Net weight: 6mg</td>
</tr>
<tr>
<td>Toxicity Statement: Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable. Attached: No</td>
</tr>
</tbody>
</table>