



**WHO International Standard
1st International Standard for Human C-peptide
NIBSC code: 13/146
Instructions for use
(Version 3.0, Dated 27/10/2015)**

1. INTENDED USE

The 1st International Reference Reagent (IRR) for human C-peptide, in ampoules coded 84/510, has been widely used for the calibration of immunoassays for human C-peptide. Stocks of the 1st IRR are exhausted, and the WHO Expert Committee on Biological Standardization (ECBS) has recognised (2010) the need for a replacement International Standard.

This reference material consists of a batch of ampoules, coded 13/146, which contain synthetic human C-peptide. The ampouled preparation has been calibrated by amino acid analysis and HPLC, and evaluated by immunoassay in an international collaborative study to determine its suitability to serve as an International Standard. A report describing the study was submitted to WHO ECBS and the proposal that this preparation is established as the 1st International Standard for human C-peptide was endorsed in November 2015.

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

8.64 µg/ampoule with expanded uncertainty of 8.21-9.07 (95% confidence; k=2.45).

4. CONTENTS

Country of origin of biological material: United Kingdom.

Each ampoule contains the residue, after freeze drying, of 0.5 ml of a solution which contained:

Synthetic C-peptide	8.64 µg
di-Sodium hydrogen phosphate anhydrous	0.41 mg
Sodium di-hydrogen phosphate monohydrate	0.29 mg
Trehalose	2.5 mg

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

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufacturers instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

For practical purposes each ampoule contains the same quantity of C-peptide. Depending on the intended use, dissolve the total contents of the ampoule in a known amount of a suitable diluent. Users should make their

own investigations into the type of diluent suitable for their own use. If extensive dilutions are prepared, a carrier protein (0.05-0.1% w/v BSA or HSA) should be added. The ampoules do not contain bacteriostat and a solution of the reagent should not be assumed to be sterile.

8. PREPARATION OF AMPOULES

Ampoules coded 13/146 containing a nominal 8.5 µg lyophilized C-peptide, sodium phosphate and trehalose were prepared according to the methods recommended for international biological standards. A weighed portion of the C-peptide was dissolved in a sterile solution containing 10mM sodium phosphate pH7.0, 0.5% (w/v) trehalose and diluted to a final volume of 1500 ml with the same buffer. This solution was passed through a filter (mean pore diameter 0.45 µm) and distributed in 0.5 ml 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.

An international collaborative study was carried out by 24 laboratories in 10 countries in three phases. Phase I of the study involved the assignment of a value to a primary calibrant in mass units by amino acid analysis and phase II applied this value to the calibration of the candidate standard, 13/146, by RP-HPLC. Laboratory estimates for 13/146 were in good agreement, and the value of 8.64 µg/ampoule, with expanded uncertainty of 8.21-9.07, was assigned from results of phase II. The results of phase II also indicated the candidate standard 13/146 was sufficiently stable, on the basis of a thermally accelerated degradation study, to serve as an International Standard.

In phase III, the candidate standard was assessed by immunoassay to determine its suitability to serve as an International Standard. The results of phase III of the collaborative study indicated that the candidate standard showed appropriate immunological activity, and results of an assessment of the commutability of the candidate standard with a small cohort of patient samples indicated that the candidate standard 13/146 is commutable with serum and urine patient samples as measured by current immunoassays. The immunoassay estimates of 13/146 were some 13% higher than the assigned content based on physicochemical assays in phases I and II. However, given that the manufacturer's assays used in this study are calibrated in terms of the IRR 84/510, this higher estimate is almost certainly a result of the limited dataset used in the original value assignment of the IRR 84/510.

Manufacturers should therefore be aware of the potential impact on their assay calibration, of the replacement of 84/510 with 13/146, which has been assigned a more accurate value using physicochemical methods.

9. STABILITY

Stability based on HPLC analysis of thermally accelerated degradation samples of the candidate standard showed a predicted yearly loss of activity when stored at -20°C of 0.07% and a predicted yearly loss of C-peptide content of 3.2% at 20°C. These results indicate that 13/146 is likely to be highly stable under long term storage conditions at -20°C and that the material will also be stable during normal shipping at ambient temperatures.

NIBSC follows the policy of WHO with respect to its reference materials. It is not the policy of the WHO 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.

10. REFERENCES



11. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to all participants in the collaborative study.

12. 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

13. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

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, white	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	
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: 3.5 mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_standardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.