Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Calcitonin, ASU1-7 Eel Calcitonin Analogue (Elcatonin)
NIBSC code: 84/614
Instructions for use
(Version 3.0, Dated 12/12/2007)

1. INTENDED USE

The International Standard consists of a batch of ampoules (coded 84/614) which was established at the 37th Meeting of the WHO Expert Committee on Biological Standardisation in 1986.

2. CAUTION

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

Human albumin batch AK 27 has been tested and found negative for HBsAg and anti-HIV antibody (Wellcozyme®). The freeze dried preparation has been tested and found negative for anti-HIV, HBsAg, anti-HCV and HCV by PCR. However, as with all materials of human origin, the preparation cannot be assumed to be free from infectious agents. The container and its contents should be used and discarded according to your own laboratory procedures. Such procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening the container to avoid cuts.

3. UNITAGE

Each ampoule of the International Standard contains 15 International Units (by definition).

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of a solution which contained:

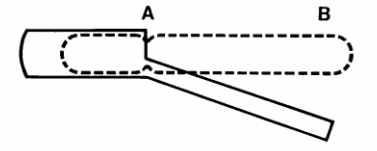
Synthetic calcitonin	approx	3µg
Human albumin	approx	250µg
Trehalose	approx	2mg

5. STORAGE

Unopened ampoules should be stored below –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 all practical purposes each ampoule of the International Standard contains the same quantity of the substances listed above. Dissolve the contents of the ampoule in a known volume of a suitable solvent (buffer at pH 4.5) with carrier protein where extensive dilution is required. No attempt should be made to weigh out any portion of the freeze dried material.
For economy of use, it is recommended that the solution be sub-divided into several small containers and stored at –40°C or below. Careful evaluation will be needed to determine a feasible time of storage.
The ampoules do not contain bacteriostat and solutions of the IS should not be assumed to be sterile.

8. PREPARATION OF AMPOULES

In 1982, 5mg of highly purified synthetic elcatonin preparation, containing 4.6% water and 4.5% acetic acid and with a stated potency of 6500 units/mg powder was donated by Toyo Jozo Company Ltd, Tokyo, Japan, through the good offices of Dr H. Yamauchi. High performance liquid chromatography carried out at NIBSC showed the material to be monocomponent and a single *in vivo* rat hypocalcaemia bioassay gave an estimate of potency of 7000 units/mg which was not significantly different from that quoted by the manufacturer. During preliminary tests on bulk material at NIBSC, it was noted that peptide was apparently lost during solution at pH 2. Albumin was added for ampouling.

9. COLLABORATIVE STUDY.

Four laboratories in three countries agreed to take part in a limited collaborative study. All participants used the intravenous dose, one hour hypocalcaemia rat bioassay. Laboratories were asked to carry out their usual assay procedure, to use freshly opened ampoules and to use a sufficient number of doses (three) so that the assumptions of linearity and parallelism could be tested, and to use a sufficient number of animals (not less than five) for each dose point.
To date the International Reference Preparation (IRP) of salmon calcitonin (sCT) (code 72/158) has been used to monitor preparations of elcatonin. To maintain continuity of a unit system, the IRP of salmon CT was included as the standard for the international collaborative study. An ampouled preparation of synthetic eel calcitonin (eCT) (code 84/587) was also included for comparison.
The potency estimate for elcatonin (84/614) in terms of the IRP sCT was homogeneous giving a weighted geometric mean of 14.4 units/ampoule (95% confidence limits 13.8-14.9) for 84/614. The assigned potency is 15 International Units. The potency estimate of eCT (84/587) in terms of the IRP sCT was also homogeneous giving a weighted mean of 43.9 units/ampoule (955 confidence limits 40-51) for 84/587.
No statistically significant deviations from parallelism were obtained but it was noted that the log dose-response for elcatonin (84/614) was consistently flatter than that for the IRP sCT in all results from two laboratories, whereas that for eCT (84/587) tended to be steeper than that for the IRP sCT.

10. STABILITY

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.
Stability of the peptide in the filling solution, kept at 4°C for 14 months, was evaluated by *in vivo* rat hypocalcaemia bioassay and no significant loss of potency was detected.
Ampoules stored at +20°C and +45°C were bioassayed after 13 months and the predicted rate of loss of potency at -20°C was estimated to be less than 1% per year.
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.

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

World Health Organization

11. REFERENCE

Annex 4, World Health Organisation Expert Committee on Biological Standardisation. Guidelines for the preparation and establishment of reference materials and reference reagents for biological substances. 29th Report, WHO Tech Rep Ser No. 626, 1978, pp101-141.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr H. Yamauchi, Toyo Jozo Company Ltd, Tokyo, Japan for providing the bulk elcatonin material for ampouling; and to all 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. 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

15. 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.

16. 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
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.	

17. 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.

18. 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: 3mg
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

efstandardsrev2004.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.