



**WHO International Standard
Glucagon, Porcine
NIBSC code: 69/194
Instructions for use
(Version 3.0, Dated 28/11/2007)**

1. INTENDED USE

This consists of a batch of ampoules (coded 69/194) which was established initially as the First International Standard for Glucagon, Porcine, for Bioassay by the WHO Expert Committee on Biological Standardization in 1973 (WHO ECBS TRS 1974). The use was subsequently extended to cover immunoassays the following year. Further details of this Standard and of its collaborative study are given in Annable et al (1974).

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

Each ampoule contains 1.49 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:

Porcine glucagon	approx	1.5 mg
Lactose AR	approx	5 mg
Sodium chloride	approx	0.25 mg
Nitrogen gas at slightly less than atmospheric pressure.		

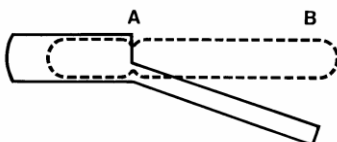
5. STORAGE

For long-term storage, it is recommended that the unopened ampoules be stored at -20°C in the dark.

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 practical purposes each ampoule contains the same amount of the same materials. Dissolve the total contents in a known amount of buffer

solution. No attempt should be made to weigh portions of the freeze-dried powder.

When glucagon is dissolved in alkaline solution at pH 11-12, it is rapidly converted into monodesamidoglucagon. The biological activity of this degradation product in rabbits is 60% of that of unconverted glucagon.

When glucagon is dissolved in acid solution to the concentration of 1mg/ml or more, it will aggregate to produce gels and ultimately insoluble fibrils.

When the contents of an ampoule are dissolved in 2ml of distilled water, the pH of the solution is about 4 and the solution remains cloudy. When the pH is lowered to 3.0, glucagon completely dissolves and can then be diluted to a convenient concentration, eg. 100µg glucagon per ml (ie. the contents of one ampoule diluted to 15ml).

It is therefore recommended that for short-term use glucagon is stored in diluted frozen solutions at concentrations about 100µg/ml and that thawing and freezing be avoided.

The material has not been sterilized and contains no bacteriostat.

PREPARATION OF AMPOULES

The batch of ampoules coded 69/194 was prepared according to the procedures used for international biological standards (WHO, 1978). 6.38g of purified porcine glucagon, kindly donated to WHO by NOVO, Denmark, was dissolved in a sterile solution containing lactose and sodium chloride. This solution was passed through a membrane filter (mean pore diameter 0.4µ) and distributed in 1.0ml aliquots into ampoules. 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.

ACTIVITY OF AMPOULE CONTENTS

The activity of the ampouled preparation was assessed in a collaborative study in which 3 laboratories in three countries participated. In total, 30 *in vivo* bioassays (in three different species) were submitted, and the assigned potency is based on the overall study mean.

8. 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.

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.

9. REFERENCES

WHO Expert Committee on Biological Standardization. 26th Report. WHO Technical Report Series No. 565, (1975) p18.

Unpublished working document, WHO/BS,74/1092.

Annable L., Bangham D.R., Salokangas A., Anneli and Storing P.L. (1974). *Acta Endoc.*, 77, 705-714.

WHO Expert Committee on Biological Standardization, 29th Report.
WEHO Technical Report Series No. 262, (1978).

10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to NOVO, Denmark, for donating the bulk glucagon.

11. 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

12. 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

13. 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.

14. 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
Other (specify):	None
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.

15. 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.

16. 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: 7mg

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.