Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Inhibin, Porcine
NIBSC code: 86/690
Instructions for use
(Version 3.0, Dated 17/12/2007)

1. INTENDED USE

This consists of a batch of ampoules coded 86/690 containing an extract of follicular fluid, which was established as the First International Standard for Inhibin, Porcine by the WHO Expert Committee on Biological Standardization (WHO ECBS) in 1990. For further details of this Standard and its collaborative study see Waites et al (1987) and Gaines-Das et al (1992).

2. CAUTION

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

The preparation contains material of human origin, which has been tested and found negative for HBsAg, HIV antibody and 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

Each ampoule contains 2000 INTERNATIONAL UNITS (by definition)

4. CONTENTS

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

Extract of porcine follicular fluid	approx	20µg
Trehalose	approx	10mg
Human plasma albumin	approx	1mg
Sodium chloride	approx	0.6mg
Acetic acid	approx	1.2mg

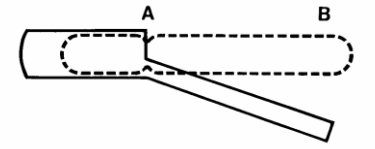
Dry nitrogen gas at slightly less than atmospheric pressure.

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.



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 all the contents in a known amount of buffer solution. No attempt should be made to weigh portions of the freeze-dried powder.
For economy of use the solution can be kept for several months if the solution is subdivided into several small containers, which are rapidly frozen to below -70°C and then stored below -30°C in the dark; repeated freezing and thawing should be avoided. If extensive dilutions are prepared, a carrier protein (0.1% w/v) should be added, which is free of peptidase.
The material has not been sterilized and contains no bacteriostat

8. PREPARATION OF AMPOULES

Bulk
This consisted of 40mg of an extract of porcine follicular fluid, batch no. WLG-4-55B, which had been prepared by procedures similar to those described by Gordon et al (1986). In brief, the inhibin was partially purified from charcoal-treated porcine follicular fluid by precipitation with acetone followed by chromatography on Sephacryl S200 and Sephadex G75. The inhibin potency of WLG-4-55B was assessed in an *in-vitro* pituitary cell assay which measured basal release of FSH. Using this assay system, the ID50 of WLG-4-55B was found to be 55ng by Dr Gordon (Houston) and 62ng by Dr de Jong (Rotterdam). The purity of this preparation is approximately 1 to 3% (w/w/).
Distribution into ampoules
In November 1986, 37.9mg of WLG-4-55B were added to 38ml of a diluent consisting of 1mM-acetic acid containing 0.1% purified peptidase-free human plasma albumin (Lister Institute, Elstree), 0.5% trehalose and 1.54mM sodium chloride at pH 4.5. Glacial acetic acid was added drop-wise to a final concentration of about 400mM in order to give an opalescent solution which was clarified using an 0.45µm filter (Millex HA, Millipore) and further diluted with the original diluent to give a concentration of 20µg of WLG-4-55B/g of solution.
The solution was then distributed into ampoules coded 86/690 (filling volume 1.0ml). The mean weight of solution in each of 32 weighted ampoules was 1.01g with a range of 0.27% of the mean. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen (WHO ECBS, 1989).

9. COLLABORATIVE STUDY.

Ten laboratories in eight countries participated in the collaborative study. Each of the participants used *in-vitro* assays, the majority of which depended upon the inhibition of release of follicle stimulating hormone from dispersed rat anterior pituitary cells. Most laboratories contributed data from two independent assays. 86/690 was compared with i) coded ampoules of 86/690 stored under conditions of accelerated thermal degradation; ii) pure 31kD bovine inhibin (code 87/534); iii) approximately 100µl of human follicular fluid (code 87/546); iv) ovine rete testis fluid protein (code 87/596); v) HPLC grade human inhibin (code 87/716).
All laboratories confirmed the activity of 86/690 used as reference preparation in the study with its previously assigned potency of 2000 units/ampoule (see summary of initial assessment, Waites et al, 1987). The thermal stability of 86/690 is satisfactory based on the consensus estimated potency of samples stored under conditions of accelerated thermal degradation.
The inherent variability in the assay systems used in the study meant that it was not possible to make any conclusions about similarities and differences seen for non-identical inhibins (Gaines Das et al, 1992).
On the basis of the study, 86/690 was deemed to be sufficiently stable and suitable to serve as a standard for *in vitro* bioassays and was established by the WHO Expert Committee on Biological Standardisation as the First International Standard for Porcine Inhibin.

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

World Health Organization

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.
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

Gaines-Das, R.E., Rose, M. and Zanelli, J.M. (1992). Initial phase in the standardization of inhibins; international collaborative study by *in vitro* bioassays of the First International Standard for Porcine Inhibin. J. Reprod. Fert., 96, 803-814.
Gordon, W.L., Liu, W.K. & Ward, D.N. (1986). Inhibin fractionation: a comparison of human and porcine follicular fluid, with particular reference to protease activation. Biol. Reprod. 35(1), 209-218.
Waites, G.M.H., Bialy, G., Gordon, W.L., Findlay, J.K., dep Jong, F.H., Robertson, D.M., Schwartz, N.B. & Storring, P.L. (1987). Proc: Serono Symposium on Inhibin-non-Steroidal Regulation of Follicle Stimulating Hormone Secretion, Tokyo, Japan. 42, 219-232. Eds. H.G. Burger, D.M. de Krester, J.K. Findlay & M. Igarashi, New York: Serono Symposia Publication from Raven Press.
WHO Expert Committee on Biological Standardization (1989). 40th Report. Guidelines for the preparation, characterization and establishment of international standards and other standards and reference reagents for biological substances. World Health Organization, Technical Report Series. No 800, p181-213.
WHO Expert Committee on Biological Standardization. (1990) 41st Report. WHO Technical Report Series. No. 814, p10-11.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to:- Dr W L Gordon and his colleagues (Houston, Texas, USA) for isolating and characterizing the bulk material; the Contraceptive Development Branch of the NIH, USA, for making the material for the standard available, through the good offices of Dr G Bialy, to Dr J K Findlay, Dr G M H Waites (WHO Special Programme of Research, Development and Research Training in Human Reproduction); Drs W L Gordon, F H de Jong (Rotterdam) and D M Robertson (Melbourne) for bioassays of the bulk and standard; to Dr P K Phillips for ampouling and to the participants in the international 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: Solid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other	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: 13mg
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_biolefstandardsrev2004.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.