WHO International Standard
Follicle Stimulating Hormone, Pituitary
NIBSC code: 83/575
Instructions for use
(Version 4.0, Dated 06/04/2013)

1. INTENDED USE
This consists of a batch of ampoules coded 83/575 which was established as the International Standard for Follicle Stimulating Hormone, Pituitary at the 37th Meeting of the WHO Expert Committee on Biological Standardization (WHO ECBS) in 1986 (WHO ECBS, 1987). For further details of this Standard and of its collaborative study see Storring and Gaines Das (1989).

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

This preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA.

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. UNTAGUE
Each ampoule contains 80 International Units (by definition).

Uncertainty: the International Unit of 83/575 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 83/575 may be considered to be the co-efficient of variation of the fill volume, which was determined to be 0.39%.

4. CONTENTS
Country of origin of biological material: United Kingdom.

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH extract</td>
<td>approx 4.17μg</td>
</tr>
<tr>
<td>Mannitol</td>
<td>approx 5mg</td>
</tr>
<tr>
<td>Human plasma albumin</td>
<td>approx 1mg</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>approx 0.6 mg</td>
</tr>
</tbody>
</table>

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 ampoure 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 quantity of the above materials. Dissolve all the contents in a known amount of buffer solution. No attempt should be made to weight portions of the freeze-dried powder.

For economy of use the solution can be kept for several months if an anti-bacterial preservative is added and the solution is subdivided into several small containers, which are frozen rapidly 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
Highly purified FSH was isolated from human pituitary glands and characterised by Professor W.R Butt and Dr S.S Lynch and their colleagues in Birmingham and generously donated to WHO. This preparation had a biological potency by in vivo bioassay (Steelman and Pohley, 1953) of greater than 15,000 IU/mg, at least as high as that found for other highly purified preparations (Storring et al. 1981). The preparation was shown to be free from significant contamination with LH and TSH.

This FSH preparation was diluted in a solution containing 0.2% (w/v) heat-treated re-purified human plasma albumin (batch AK1, Lister Institute, Elstree), 1 (w/v) mannitol and 15.4mM sodium chloride. The solution was distributed into ampoules coded 83/575 as approximately 0.5ml aliquots. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen (Campbell, 1974; WHO ECBS, 1978). The batch consisted of 3872 ampoules. The mean weight of solution in each of 80 weighed ampoules was 0.503g with a range as % of mean of 0.78%.

9. COLLABORATIVE STUDY AND THE ASSIGNMENT OF UN
The collaborative study was carried out by 27 laboratories in 13 countries, in which the preparation of FSH in ampoules coded 83/575 was assayed against the second International Reference Preparation of FSH and LH, Pituitary, for Bioassay (in ampoules coded 78/549).

Estimates of the biological potency of this material by the in vivo assay method (based on the increase of ovarian weight in HCG treated immature rats) gave a mean value of about 80 IU/ampoule; estimates with in vitro bioassay and hormone receptor-binding assay systems gave heterogeneous values which varied between 5 and 31 IU/ampoule with a mean of about 16 IU/ampoule. In accordance with its policy (WHO ECBS 1982), the WHO Expert Committee on Biological Standardisation agreed that the potency assigned to the new standard should be made on the basis of the results of the in vivo bioassays (WHO, ECBS 1987). Such assays reflect the ability of FSH glycoprotein isohormones and their metabolites to exert their full natural in-situ biological actions.

The committee recognized, however, that the introduction of the new International Standard, with a defined potency of 80 IU/ampoule will...
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezed dried powder</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>
</tbody>
</table>

Other (specify): contains material of human origin

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: 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_biolrefstandardsrev2004.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.