



**WHO International Standard
Luteinizing Hormone, Pituitary, alpha subunit
NIBSC code: 78/554
Instructions for use
(Version 4.0, Dated 28/03/2013)**

1. INTENDED USE

This consists of a batch of ampoules (coded 78/554) which was established by WHO in 1984⁽¹⁾.

2. CAUTION

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

The 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 preparations of human origin, this material 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 contains 10 International Units (by definition). (This unitage was assigned on the basis that the International Unit is represented by 1 microgram of the pure subunit preparation).

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:

Alpha subunit of pituitary LH (LH-alpha)	approx 10 µg
Lactose	" 2.5 mg
Human plasma albumin	" 0.5 mg
Sodium chloride	" 45 µg

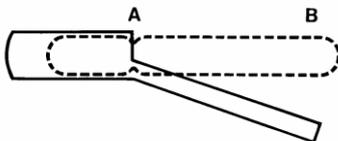
Nitrogen gas at slightly less than atmospheric pressure.

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 an antibacterial 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

Bulk Material

Some 20mg of LH-alpha (batch NM14 DEAE II) was prepared for WHO by Drs G. Hennen and R.M Lequin and their colleagues by the method of Closset et al⁽²⁾. From LH batch NM14 (Loeber⁽³⁾). This subunit preparation was characterised by them using amino acid analysis, chromatography and immunoassay; its contamination with the beta subunit of human pituitary LH (LH-beta) was found to be < 0.5% (w/w) in two different immunoassay systems specific for the LH-beta. The LH potencies (with 95% confidence limits) of the LH (batch NM14) from which the LH-alpha was prepared, were found by Loeber⁽³⁾ to be 8400 (6630-11100) IU/mg by ovarian ascorbate depletion assay⁽⁴⁾ and 2360 (1560-3210) IU/mg by seminal vesicle weight gain assay⁽⁵⁾, each in terms of the first IRP of Human Pituitary Gonadotrophins (FSH and LH) for Bioassay (coded 69/014)^(6,7). (See Storrington et al⁽⁸⁾, for a comparison of these biological potencies with those of other preparations of highly purified human pituitary LH).

Distribution into ampoules.

In May 1978, 5.94 mg of the preparation of LH-alpha was dissolved in water containing 0.5% (w/v) lactose and 0.1% (w/v) purified human albumin (by dilution of a 10% solution in 0.89% sodium chloride (batch AK8; Lister Institute, Elstree)), to a final concentration of about 20 micrograms LH-alpha/ml. Equal volumes (0.5ml) of this solution were distributed into ampoules and the ampoule contents freeze-dried, secondarily desiccated and sealed under nitrogen as described by Campbell⁽⁹⁾ and the WHO Expert Committee on Biological Standardisation⁽¹⁰⁾. The batch consisted of 506 ampoules. The mean weight of filling solution in each of 11 weighed ampoules was 0.504mg, with a range of 0.19% of the mean.

9. CONTAMINATING ACTIVITIES

The LH activity (with 95% confidence limits) of the ampoule contents was estimated to be 0.511 (0.052-1.74) IU/ampoule by ovarian ascorbate deletion assay in terms of the IRP coded 69/014, and to be 0.088 IU/ampoule by in vitro bioassay⁽¹¹⁾ in terms of the Second IRP of Human Pituitary FSH and LH for Bioassay^(7,12). Assuming a potency of 10000 IU/mg for highly purified LH⁽⁶⁾, these activities would represent at most a contamination of 0.5% (w/w) with native LH. In specific immunoassay systems, the ampoule LH-alpha preparation showed cross reactions of: 0.06% (w/w) with FSH; 0.12% (w/w) with TSH; 0.64% (w/w) with LH-beta; 1.96% (w/w) with LH in one assay system and 11.1% (w/w) in another which however there was significant (p < 0.011) deviation from parallelism between the log dose-response curves.

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. Unopened ampoules 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.

11. REFERENCES

1. WHO Expert Committee on Biological Standardisation (1985). WHO Tech Rep Ser No 725.
2. Closset H, Vandalem J-L, Hennen G and RM Lequin (1975) Eur J Biochem 57, 325.
3. Loeber JG (1977) Acta Endocrinol 85, Suppl 210
4. Parlow AF (1961) In: Human Pituitary Gonadotrophins, p300, Ed. A Albert, Charles C Thomas, Springfield, Illinois.
5. Van Hell H, Matthijsen R & GA Overbeek (1964) Acta Endocrinol 47, 409
6. WHO Expert Committee on Biological Standardisation (1975). WHO Tech Rep Ser No 565.
7. Bangham DR, Berryman I, Burger H, Cotes PM, Furnival BE, Hunter WM, Midgley AR, Mussett MV, Reichert LE Jr, Rosemberg RJ, Ryan RJ & Wide L (1973) J Clin Endocrinol 101, 339
8. Storrington PL, Zaidi AA, Mistry YG, Lindberg M, Stenning BE & E Diczfalusy (1982) Acta Endocrinol 101, 339
9. Campbell PJ (1974) J Biol Standardisation 2, 259.
10. WHO Expert Committee on Biological Standardisation (1978). WHO Tech Rep Ser No 626.
11. Van Damme M-P, Robertson DM & Diczfalusy E (1974) Acta Endocrinol 77, 655
12. WHO Expert Committee on Biological Standardisation (1981). WHO Tech Rep Ser No 658.

12. ACKNOWLEDGEMENTS

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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>
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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

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

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 (specify): 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_biologicalstandardsrev2004.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.