

WHO International Standard Luteinizing Hormone, Human, Recombinant NIBSC code: 96/602 Instructions for use (Version 6.0, Dated 24/09/2025)

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1. INTENDED USE

The WHO International Standard for Luteinizing Hormone (LH), coded 96/602, is intended for the calibration of preparations of recombinant DNA-derived LH (INN; Lutropin Alfa) by bioassay. It was established by the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) at its 54th meeting in 2003 [1].

LH is a complex glycoprotein, in which glycosylation causes an inverse correlation between in vivo and in vitro potencies. Furthermore, differences in glycosylation profiles between LH preparations may lead to different relationships between in vivo and in vitro potencies. Consequently, this product is primarily intended for calibration of LH preparations by in vivo bioassay. Whilst the product is expected to show potency in in vitro (e.g. cell-based) bioassays, its use for calibration of such assays should be approached with caution. This is because the LH source material contained in this product may not be structurally equivalent (in terms of glycosylation profile) to the LH preparation to be tested, and in such circumstances would not be a suitable calibrator of an in vitro bioassay [2].

Please note, this product is assigned a potency value in International Units (IU) only (see Section 3). The approximate content in mass units provided in Section 4 has not been experimentally derived and should not be used as a formal mass value assignment for calibration purposes under any circumstances. Assignment of a mass value to this product would serve to define a specific potency (in IU per mg) for LH, which must be avoided as the specific potency may differ between LH preparations due to variable glycosylation.

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. 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 of 96/602 contains 189 International Units (IU) per ampoule (by definition).

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content, and was experimentally determined as $\pm\,0.65\%$ (cv)

4. CONTENTS

Country of origin of biological material: United Kingdom. Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution that contained:

Recombinant luteinizing hormone approx. 8.8µg
Human plasma albumin approx. 2mg
Lactose approx. 10mg
Sodium chloride approx. 8.9mg

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

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

For practical purposes each ampoule contains the same quantity of luteinizing hormone. The entire content of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. No attempt should be made to weigh out portions of the freeze dried powder. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

8. PREPARATION OF AMPOULES

Bulk rLH: This consisted of approximately 118mg of highly purified rLH (Batch no BLBA9410), synthesized in Chinese hamster ovary cells, and generously donated to WHO by Serono, through the good offices of Dr A. Eshkol. It was received as seven 2-ml aliquots of a frozen solution stated to contain 8.42 mg/ml of rLH at a stated specific activity of 17,760 IU/mg, as estimated by the seminal vesicle weight gain bioassay (Van Hell et al., 1964). Analyses by Serono showed that the identity and purity of this batch of rLH as assessed by SDS-PAGE, isoelectric focusing and size-exclusion HPLC conformed to those of the Serono in-house interim reference preparation.

Distribution into ampoules: LH 96/602 was prepared in September 1996. Some 3.66ml of the bulk solution from two aliquots containing 30.8mg rLH, were diluted to a volume of 3500ml in a solution containing 1%(w/v) lactose, 0.89%(w/v) sodium chloride and 0.2%(w/v) human plasma albumin (Zenalb, batch no ABC 0183, Bio Products Laboratory, Elstree). The solution was distributed into ampoules as approximately 1.0ml aliquots. The solution of rLH was kept at 4°C throughout. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen (Campbell, 1974; WHO ECBS 1990). The batch consisted of 3401 ampoules. The mean weight of filling solution in 67 weighed ampoules was found to be 1.0072g with a coefficient of variation of 0.65% and a range as % of the mean of 1.01.

9. COLLABORATIVE STUDY

The candidate rLH preparation 96/602 was evaluated by bioassay against current LH reference preparations in an international collaborative study by 9 laboratories in 9 countries using the seminal weight gain LH



bioassay. The LH preparations in the study were also compared by one laboratory in one LH immunoassay system. The objectives of the study were thus:

- to compare by LH bioassay the proposed IS with the fourth International Standard for Human Urinary FSH and LH (IS 98/704) and the second International Standard for Human Pituitary LH (IS 80/552);
- to calibrate the proposed IS by in-vivo LH bioassay in terms of the IS 98/704 and the IS 80/552;
- to estimate the LH bioactivities of accelerated thermal degradation samples of the proposed IS, so as to assess the stability of the proposed IS.

9.1 Activity of ampoule contents

The main function of preparation 96/602 is to serve as an international standard for recombinant LH for bioassay. It was recommended that the value determined relative to the urinary LH standard IS 98/704 be adopted to ensure continuity of the international unit of urinary LH which is currently used to calibrate LH-containing therapeutic products. On this basis, the Expert Committee on Biological Standardization of the World Health Organization established the preparation, in ampoules coded 96/602, as the First International Standard for Luteinizing Hormone, Human, Recombinant and assigned an activity to it of 189 International Units per ampoule.8

9.2 Stability

Combining the results of accelerated degradation studies on samples held at elevated temperatures for more than five years gave a predicted yearly loss of activity for samples stored at -20°C of less than 0.01%. These data indicate that preparation 96/602 is sufficiently stable to serve as an international standard.

10. STABILITY

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, temperaturecontrolled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact standards@nibsc.ac.uk..

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.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

11. REFERENCES

- WHO/BS/03.1983
- B. Cowper, M. Moore, C. Burns. Therapeutic glycoprotein hormones: Considerations for the use of in vitro alternatives to in vivo potency assays (2025). J. Pharm. Biomed. Anal. 262, 116895.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the following: the participants in the international collaborative study; Serono (through the good offices of Dr A Eshkol) for providing the bulk rLH; Mr P Gerson, Mrs J Longley, Ms P Lloyd and Mr RJ Tiplady for preliminary bioassays; Dr MP Rose and Dr P Dawson for ampouling; and to Ms G Creeber for data entry.

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 (FC) No 1272/2008: Not applicable or not classified

(EC) NO 1272/2008: NOT applicable of not classified		
Physical and Chemical properties		
Physical	Corrosive:	No
appearance: Freeze		
dried powder		
Stable:	Oxidising:	No
Yes		
Hygroscopic:	Irritant:	No
Yes		
Flammable:	Handling:	See caution, Section 2
No		
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: Se	eek 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.

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 freezedrying.

Net weight: 21mg

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable.

Attached: No

19. 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 https://www.who.int/publications/m/item/annex2trs932(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.

