



**WHO International Standard
Luteinizing Hormone, Human, Recombinant
NIBSC code: 96/602
Instructions for use
(Version 4.0, Dated 18/01/2008)**

1. INTENDED USE

This consists of a batch of ampoules, coded 96/602, containing recombinant luteinizing hormone (rLH) synthesized in Chinese hamster ovary cells (rINN: lutropin alfa). The preparation was examined by international collaborative study and established as the First International Standard for Luteinizing Hormone, Human, Recombinant by the Expert Committee on Biological Standardization of the World Health Organization in November 2003. The main function of 96/602 is to serve as an international standard for the bioassay of therapeutic recombinant LH products.

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. 8.8 μ g
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.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is

pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

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, temperature-controlled 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

1. Van Hell H, Matthijsen R & Overbeek GA (1964). Effects of human menopausal gonadotrophin preparations in different bioassay methods. *Acta Endocrinologica* 47: 409-418.

2. Campbell PJ (1974). International biological standards and reference preparations. II. Procedures used for the production of biological standards and reference preparations. *Journal of Biological Standardization* 2:269-272.

3. WHO Technical Report Series No.800, 1990 181-214

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

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

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: Freeze dried powder	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: 21mg
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.