



**WHO Reference Reagent
Prolactin, Human, recombinant
NIBSC code: 97/714
Instructions for use
(Version 2.0, Dated 16/01/2008)**

1. INTENDED USE

This consists of a batch of ampoules (coded 97/714) containing recombinant human prolactin expressed in murine C127 cells (1). The preparation was analysed by international collaborative study and established as the First WHO Reference Reagent for Prolactin, Human, Recombinant, for Immunoassay by the Expert Committee on Biological Standardization of the World Health Organisation in November 2001. The primary use of the preparation, along with its glycosylated (98/580) and non-glycosylated (98/582) components, is in investigating and characterizing the specificity of existing prolactin assays, and in particular, the suitability of using recombinant prolactin as an immunoassay standard.

This preparation is not intended to replace the 3rd International Standard for prolactin, 84/500, which remains the primary standard for calibration of diagnostic immunoassays for prolactin.

2. CAUTION

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

The material is not of human or bovine origin. 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 97/714 contains 24.5µg recombinant prolactin (by definition).

On the basis of bioassay results, preparation 97/714 may be assumed to contain 1400 mU per ampoule.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1ml of a solution that contained:

4.5mg NaCl	3.0mg arginine
4.0mg Na phosphate	0.1mg Tween 20
30.0mg trehalose	recombinant PRL

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 prolactin. The entire content of each ampoule should be completely dissolved in an accurately measured amount of diluent, such as PBS, saline or assay diluent with 0.05-0.1% carrier protein. 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.

8.1 Bulk material

A preparation of prolactin (>99% pure by RP-HPLC), expressed in murine C127 cells and consisting of approximately 20% glycosylated and 80% non-glycosylated components (1), was kindly donated to WHO by Genzyme Corporation, Framingham, MA, USA.

8.2 Distribution into ampoules

The material was received as a frozen solution at 0.545 mg/ml in 25mM HEPES/150 mM NaCl pH 8, which, after dilution in a solution containing 0.45%(w/v) sodium chloride, 0.4% (w/v) sodium phosphate, 3.0% (w/v) trehalose, 0.3% (w/v) arginine and 0.01% Tween 20, pH 6.99, was distributed into ampoules as 1ml aliquots, lyophilised and sealed according to procedures described by WHO for International Biological Standards (2) and stored at -20°C in the dark.

9. COLLABORATIVE STUDY

The preparation 97/714, along with its glycosylated (98/580) and non-glycosylated components (98/582), was evaluated in an international collaborative study in which fifteen laboratories in eight countries took part. Assays contributed included in vitro assays mostly based upon proliferation in the Nb2 cell-line, competitive and non-competitive immunoassays and SE-HPLC and RP-HPLC. The study was designed to:

- compare, by immunoassay and bioassay, the ampouled preparations of rhPRL with local standards presently in use,
- calibrate the preparations of rhPRL for use as potential reference reagents,
- assess the stability of the proposed reference reagents after accelerated thermal degradation,
- assess the ampoule contents of rhPRL and its components by physico-chemical methods
- characterize assay systems which may discriminate between the glycosylated and non-glycosylated components,
- provide evidence of commutability of the assay systems when calibrated against a recombinant preparation.

9.1 Activity of ampoule contents

The primary function of preparation 97/714 is to serve as a standard for immunoassay and on the basis of the HPLC results, it was established as the First WHO Reference Reagent for Prolactin, Recombinant, Human, with a defined content of 24.5µg per ampoule. For the purposes of bioassay calibration, the preparation coded 97/714 may be assumed to contain 1400 mU per ampoule.

10. STABILITY

Combining the results of accelerated degradation studies by all assay methods a predicted loss of ~0.01% per year at -20°C was calculated,



indicating that the ampouled preparation 97/714 is sufficiently stable to serve as reference reagent.

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

- Price AE, Kimberly B, Higgins EA, Cole ES and Richards SM. 1995 Studies on the microheterogeneity and in vitro activity of glycosylated and non-glycosylated recombinant human prolactin separated using a novel purification process. *Endocrinology* 136:4827-4833.
- WHO Technical Report Series No.800, 1990 181-214

12. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants. The preparation of recombinant prolactin for ampouling was generously provided to WHO by Genzyme Corporation, Framingham, MA, USA. We also thank the staff of Standards Division for preparation of the ampouled materials.

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.

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 WHO International Laboratory for Biological Standards,
 UK Official Medicines Control Laboratory

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): Can react with oxidising materials. Avoid contact with acids and alkalis.	
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: 42mg
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.