Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Prolactin (Human, Recombinant, Non-Glycosylated) For Immunoassay
NIBSC code: 98/582
Instructions for use
(Version 3.0, Dated 14/01/2008)

1. INTENDED USE

This consists of a batch of ampoules (coded 98/582) containing the non-glycosylated component of 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, Non-Glycosylated, for Immunoassay by the Expert Committee on Biological Standardization of the World Health Organisation in November 2001. The primary use of this preparation, along with the parent recombinant prolactin material (97/714) and the glycosylated component (98/580), is for investigating and characterizing the specificity of existing prolactin assays.

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 98/582 contains 10.5µg recombinant non-glycosylated prolactin (by definition). On the basis of bioassay results, preparation 98/582 may be assumed to contain 670 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 (non-glycosylated)	

5. STORAGE

Unopened ampoules should be stored at –20ºC
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 non-glycosylated prolactin. 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

8.1 Bulk material

A preparation of non-glycosylated prolactin, purified (>95% pure by SDS-PAGE) from recombinant prolactin expressed in murine C127 cells (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 1.24 mg/ml in 25mM HEPES pH 8.0, 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 98/582, along with the parent recombinant prolactin preparation (97/714) and the glycosylated component (98/580), 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 98/582 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, Non-Glycosylated with a defined content of 10.5µg per ampoule. For the purposes of bioassay calibration, the preparation coded 98/582 may be assumed to contain 670 mU per ampoule.

8. 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.
In accelerated degradation studies, the relative potencies of ampoules of 98/582 at +20°C, +37°C and +45°C were similar to those for recombinant prolactin 97/714 indicating an equivalent level of stability. This suggests a predicted loss of ~0.01% per year, indicating that the ampouled preparation 98/582 is sufficiently stable to serve as reference reagent.

9. REFERENCES

1. 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.
2. WHO Technical Report Series No.800, 1990 181-214

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

World Health Organization

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants. The preparation of recombinant non-glycosylated 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.

11. 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

12. 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

13. 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.

14. 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.

15. 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.

16. 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: 42 mg
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_biolefstandardsrev2004.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.