



**WHO Reference Reagent
Thyroid-Stimulating Hormone (TSH), Recombinant, Human
NIBSC code: 94/674
Instructions for use
(Version 3.0, Dated 21/01/2008)**

1. INTENDED USE

This consists of a batch of ampoules (coded 94/674) containing recombinant DNA-derived human thyroid-stimulating hormone which was established as the First WHO Reference Reagent for Thyroid-Stimulating Hormone, Recombinant, Human by the Expert Committee on Biological Standardization of the World Health Organisation at its forty-seventh meeting in 1996 (1). The WHO Reference Reagent for rDNA-derived TSH is intended for use in the validation of assay performance by the characterisation of systems which may distinguish between TSH from different sources (2). **This preparation is not intended to replace the Third IS for TSH (81/565).**

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

This preparation has been assigned a unitage of 6.7 milli Units (or 0.0067 Units) 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 which contained:

Recombinant DNA-derived TSH	nominally 1µg
Human plasma albumin	0.1% v/v
Lactose	5mg

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 manufacturers instructions provided with the ampoule breaker.

7. USE OF MATERIAL

For practical purposes each ampoule contains the same quantity of TSH. The entire contents 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 since results have indicated that under these conditions the recovery of material from the ampoule may be inconsistent with the assigned ampoule content. The material has not been sterilized and the ampoules contain no bacteriostat.

8. PREPARATION OF AMPOULES

8.1 Bulk Material

Highly purified (>95% by SDS-PAGE) rDNA-derived TSH, expressed in Chinese hamster ovary (CHO) cells (3), was kindly donated to WHO by the Genzyme Corporation, Framingham, MA, USA through the good offices of Dr M Luker, Genzyme, West Malling, Kent, UK.

8.2 Distribution into ampoules

The preparation was received as a lyophilised white powder which, after dilution in a solution containing 0.1% (w/v) human serum albumin and 0.5% (w/v) lactose, was distributed into ampoules as 1.0ml aliquots.

The ampouled preparation was then lyophilised and sealed according to procedures described by WHO for International Biological Standards (4) and stored at -20°C in the dark. Each ampoule (coded 94/674) has a nominal content of 1µg TSH, 1mg human plasma albumin and 5mg lactose. The preparation consists of a batch of 1969 ampoules and the mean weight of solution in 43 weighed ampoules was 1.0033g with a coefficient of variation of 0.175%.

9. COLLABORATIVE STUDY

The preparation in ampoules coded 94/674 was evaluated by international collaborative study in which thirty-three laboratories in eleven countries took part. The study was designed to:-

- compare rDNA TSH and pituitary TSH in a variety of immunoassay systems.

- assess the suitability of the candidate preparation of rDNA TSH to serve as a reference reagent for the calibration of diagnostic immunoassays.

- assess the stability of the candidate preparation of rDNA TSH after accelerated thermal degradation.

- confirm the bioactivity of rDNA TSH after ampouling and lyophilization procedures.

9.1 Estimate of immunoactivity

The weighted geometric mean of the estimates of 94/674 in terms of the Second Reference Preparation of Pituitary TSH was 6.70 milli units per ampoule with 95% confidence limits of 6.38 to 7.02, n=35.

9.2 Estimate of biological activity

The ampouled preparation 94/674 was found to have appropriate biological activity in bioassays in five laboratories, but there were insufficient data for reliable calibration by bioassay.

9.3 Assigned unitage

The study showed differences in discrimination between pituitary and recombinant TSH by different assay systems, indicating that the recombinant TSH is unsuitable to serve as a primary standard for all TSH immunoassays. The assigned potency of 0.0067 milli Units per ampoule of the WHO Reference Reagent preserves the continuity of the IU but it cannot be guaranteed without validation of the individual assay system.

10. STABILITY

Based on the combined estimates from the immunoassays, the predicted loss of immunoactivity for ampoules of 94/674 stored continuously at -20°C is less than 0.1% per year.



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. 47th Report, 1996. WHO Expert Committee on Biological Standardization.
2. Rafferty B. & Gaines Das R. Comparison of pituitary and recombinant human thyroid-stimulating hormone (rhTSH) in a multicentre collaborative study: establishment of the First WHO Reference Reagent for rhTSH. Clin Chem 1999; 45: 2207-2215
3. Recombinant human thyroid stimulating hormone: development of a biotechnology product for detection of metastatic lesions of thyroid carcinoma. Cole ES, Lee K, Lauziere K, Kelton C, Chappel S, Weintraub B, Ferrara D, Peterson P, Bernasconi R, Edmunds T, et al. Biotechnology 1993 11: 1014-24
4. WHO Tech Rep Ser No 800, 1990 181-214

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to: Dr M Luker and the Genzyme Corporation for providing the material; Dr P Dawson and the staff of Standards Processing Division for ampouling the preparation; and the participants in the collaborative study.

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

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

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: 6mg
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.