WHO International Standard
Thyroid Stimulating Hormone, Human, for Immunoassay

NIBSC code: 81/565
Instructions for use
(Version 6.0, Dated 08/04/2015)

1. INTENDED USE
This consists of a batch of ampoules, coded 81/565, containing thyroid-stimulating hormone derived from the same pituitary extract as the 2\textsuperscript{nd} IRP for TSH 80/558, stocks of which are now exhausted. The preparation was examined by international collaborative study and established as the 3\textsuperscript{rd} International Standard for Thyroid-Stimulating Hormone, Human, for Immunoassay by the Expert Committee on Biological Standardization of the World Health Organization in November 2003.

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 probably will 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 81/565 contains 11.5 mIU per ampoule (by definition). Uncertainty: the assigned unitage is arbitrary and 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 measured as ± 0.38% (cv).

4. CONTENTS
Each ampoule contains the residue after freeze-drying of 0.5 ml of a solution that contained:

- Purified human pituitary TSH extract nominally 2 µg TSH
- Peptidase-free human serum albumin 1mg
- Lactose 5 mg

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

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or end.

Place the ampoule in the ampoule opener, positioning the score at position ‘A’: shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point ‘B’. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.

Side view of ampoule opening device containing an ampoule positioned ready to open. ‘A’ is the score mark and ‘B’ the point of applied pressure.

7. USE OF MATERIAL
For practical purposes each ampoule contains the same quantity of thyroid-stimulating 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 AMPULES
A quantity of highly purified extract was prepared and donated by Dr AF Parlow (Harbor-UCLA Medical Centre, Torrance, CA, USA; parlow@humc.edu) through the National Hormone and Pituitary Program (NHPP) of the USA. Ampouling, lyophilisation and sealing of the pituitary TSH preparation 81/565 were carried out according to procedures laid down by WHO for International Biological Standards (WHO 1978) and have been described previously (Gaines Das & Bristow 1985). Briefly, purified pituitary TSH extract was dissolved in a sterile solution containing 0.2% (w/v) peptidase-free human serum albumin and 1% (w/v) lactose. The solution was passed through a membrane filter (0.45µm) and distributed in 0.5 ml aliquots into ampoules. Check weights were carried out during the filling procedure as an estimate of variance in ampoule content and the residual moisture content of the lyophilised material was also determined. These were 0.502 g (CV 0.38%) and 0.08% respectively.

9. COLLABORATIVE STUDY
The candidate pituitary TSH preparation 81/565, along with other pituitary TSH preparations, was evaluated in an international collaborative study in which nine laboratories in six countries took part. Assays contributed were all immunoassays except for a single in vitro assay based on CAMP release from bovine thyroid membranes. The study was designed to:
- compare by immunoassay the ampouled preparations of TSH with local standards presently in use,
- confirm the calibration of the candidate preparation of TSH for use as a potential International Standard,
- confirm the activity of the candidate preparation relative to the original study preparations,
- assess the stability of the candidate preparation using thermally accelerated degradation samples,
- compare rDNA TSH and pituitary TSH in a variety of immunoassay systems.

9.1 Activity of ampoule contents
The main function of preparation 81/565 is to serve as a primary reference reagent against which secondary standards for immunoassay of TSH are calibrated. On the basis of the immunoassay results from the collaborative study, 81/565 was established by the ECBS of WHO as the 3\textsuperscript{rd} International Standard for TSH. Human, for Immunoassay with a defined content of 11.5 mIU per ampoule. This preparation replaces the 2\textsuperscript{nd} IRP for TSH, 80/558. All attempts have been made to ensure the continuity of the unit as evidenced by the results from the majority of assay systems in the study. However this cannot be guaranteed for all assay systems and recalibration may be necessary.

9.2 Stability
Combining the results of accelerated degradation studies by all assay methods a predicted loss of 0.04% per year at -20°C was calculated, indicating that the ampouled preparation 81/565 is sufficiently stable to serve as reference reagent.

10. STABILITY
NIBSC follows the policy of WHO with respect to its reference materials. 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, where known, the appropriate NIBSC scientist. In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

11. REFERENCES
1. WHO Tech Rep Ser No 626, 1978

12. FURTHER INFORMATION
Further information can be obtained as follows:
This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
Derivation of International Units: http://www.nibsc.org/standardisation/international_standards.aspx
NIBSC Terms & Conditions: http://www.nibsc.org/terms_and_conditions.aspx

13. 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

14. 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.

15. MATERIAL SAFETY SHEET

16. 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.

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17. INFORMATION FOR CUSTOMS USE ONLY

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_biol
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.