



**WHO International Standard
2nd International Standard for Thyroid Stimulating Antibody
NIBSC code: 08/204
Instructions for use
(Version 2.0, Dated 28/03/2013)**

1. INTENDED USE

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2006) the need for a replacement International Standard for Thyroid-stimulating antibody (TSAb) for the calibration of TSAb assays. The 2nd IS, coded 08/204 was established at the 61st Meeting of the ECBS. This material replaces the 1st IS coded 90/672, which is discontinued.

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 HIV, Hepatitis C and Hepatitis B. 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 contains 113 milli INTERNATIONAL UNITS (mIU)

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1ml of normal human serum containing:

monoclonal Thyroid-stimulating antibody	nominally 1µg.
HEPES buffer	40mM

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

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

For practical purposes each ampoule contains the same quantity of TSAb. The entire content of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

COLLABORATIVE STUDY

The preparation was evaluated in a collaborative study in which thirteen laboratories in six countries took part, organised with the following aims:

1) To calibrate the candidate standard (08/204) relative to the 1st IS for TSAb (90/672).

3) To demonstrate the suitability of the preparation 08/204 to serve as the 2nd IS for TSAb by examining its behaviour in receptor binding assays and bioassay systems.

3) To determine the stability of the preparation 08/204 by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study.

The 1st IS consisted of freeze-dried plasma proteins from a single human patient with high TSAb levels who was pregnant and whose plasma was regularly exchanged by plasmapheresis during pregnancy. Since it has proved impossible to identify a similar source and volume of plasma or serum with high autoantibody titre for the preparation of a replacement international standard, the candidate standard (coded 08/204) prepared and calibrated in this study is a human monoclonal thyroid stimulating autoantibody.

Analysis of the fitted slopes for the dose-response lines of 08/204 and 90/672 in receptor binding assays and bioassays showed no consistent slope differences, demonstrating that the candidate standard 08/204 fulfills the requirement of a replacement international standard in terms of parallelism of assay response with this plasma preparation of autoantibodies from a patient with Graves' disease.

The geometric mean potency of 08/204 from receptor binding assays alone is 0.113 IU per ampoule (n=21; 95% confidence limits 0.106 – 0.120). The geometric mean potency calculated from bioassays alone is 0.242 IU per ampoule (n=4; 95% confidence limits 0.166 – 0.353) which is significantly higher than the potency calculated by receptor binding assays and would exclude the combination of results from both methods. These data also suggest that the candidate standard 08/204 is approximately two-fold more potent in these bioassays than the 1st IS. This is perhaps not surprising since the candidate standard is known to consist solely of thyroid stimulatory immunoglobulins, rather than the combination of both thyroid stimulatory and thyroid inhibitory immunoglobulins present in the 1st IS. As a result, the establishment of 08/204 as the 2nd IS for TSAb would not represent a formal continuity of unitage for the calibration of bioassays. It is worth noting that this is not a problem encountered solely as a result of the use of a monoclonal TSAb as a candidate standard, since it is also highly likely that the functional heterogeneity of patient autoantibodies would mean that any two plasma- or serum-derived preparations, including pooled patient materials, would have different potencies in functional TRAb bioassays. Taking these data together with the finding that the between-laboratory variability is also greater for bioassays (GCV 26.8%) compared to receptor binding assays (GCV 14.5%), the candidate standard has been value-assigned on the basis of receptor binding assays alone with an assigned unitage of 0.113 IU per ampoule which will reflect formal continuity of unitage with 90/672 for these assays alone. This is unlikely to be a significant restriction to users of bioassays since these are more commonly reported as a percentage increase over reference control serum rather than in terms of the 1st IS.

Finally, it should also be noted that the monoclonal nature of the preparation 08/204 may render it unsuitable as an experimental standard for TSAb in some basic research milieu since it may not truly reflect the action of a mixed population of thyroid-stimulating antibodies in these settings.

The candidate preparation 08/204 appears to be sufficiently stable to serve as an International Standard since the predicted yearly loss of TSAb potency at -20°C is 0.018% based on the thermally accelerated degradation samples assayed in this study. These results indicate that 08/204 is likely to be highly stable under long terms storage conditions at -20°C.

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.



Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

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. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants.

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: White 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: 12mg
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_biol_standardsrev2004.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.