



**WHO International Standard
WHO 2nd International Standard for Thrombin 01/580 US
FDA/CBER Thrombin Standard Lot K
NIBSC code: 01/580
Instructions for use
(Version 4.0, Dated 31/10/2016)**

1. INTENDED USE

The above named Standard has been developed to replace the 1st International Standard for Alpha Thrombin (89/588) and the US Standard Thrombin, Lot J. The potency of the new Standard is 110 IU/ampoule which is equivalent to 110 US units/ampoule.

Low stocks of two international standards for Thrombin (the WHO International Standard for Alpha Thrombin, 89/588, and the US Standard Thrombin, lot J) necessitated development of a replacement preparation. Replacement of both Standards presented an opportunity to develop a joint Standard with a common unit, thus eliminating problems caused by the small but assay-dependent difference between the existing units and Standards (the IU in the case of the International Standard and the US unit in the case of the US Standard, also commonly referred to as the "NIH Unit"). A collaborative study was organized and involved 25 laboratories from 15 countries worldwide (1). Both the International Standard (89/588) and US Standard, lot J, were included along with two candidate Thrombin preparations donated by manufacturers. On the basis of results from this study, one of these preparations, 01/580, was chosen to be the replacement Standard and is designated the WHO 2nd International Standard for Thrombin, 01/580, and the US FDA/CBER Thrombin Standard, Lot K. The International Standard was established by the Expert Committee on Biological Standardisation of the WHO in February 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 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

Participants in the collaborative study to calibrate the replacement thrombin standard were requested to perform their in-house methods for thrombin potency determinations using their usual reagents. Potency determinations could be performed using fibrinogen or plasma substrates or alternatively using a chromogenic method. Participants were encouraged to perform more than one method if possible. The potency of the candidate materials was calculated using both the International Standard and US Standard as reference standard. Further analysis was also performed to determine if there was any effect on results of the different substrates used: human fibrinogen, bovine fibrinogen, plasma and chromogenic substrates. The conclusion of the study was that candidate material D, coded 01/580, was a suitable replacement standard for both the International Standard and US Standards. The potency of this material is 110 IU/ampoule. This figure was calculated using results from all clotting assays using human or bovine fibrinogen or plasma. Results from chromogenic assays were not used in the calculation of this value.

Uncertainty: the assigned unitage 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 determined to be +/- 0.07%.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The purified thrombin used to make the Standard was generously provided by a manufacturer as a purified solution of human thrombin prepared from pooled plasma with a specific activity of 2000-3000 IU/mg. The material was shipped to NIBSC as a frozen solution where it was then thawed and diluted to a concentration of approximately 100 IU/ml in a solution of 10 mM HEPES, pH 7.4, containing 0.15M NaCl and 5 mg/ml human albumin solution (with low protease activity).

This solution was dispensed into ampoules in 1 ml aliquots. The filling operation gave a total of 9676 ampoules with a mean filling weight of 1.0061g (cv = 0.07%). After freeze drying the ampoules had a mean dry weight of 0.0184g (cv = 1.13%) and a residual moisture of 0.17% (cv = 12.4%). The alpha thrombin content of the Thrombin Standard is not known exactly but the international collaborative study demonstrated by the ratio of clotting to chromogenic activity that it is very similar to the previous 1st International Standard for Alpha Thrombin, 89/588 (1).

5. STORAGE

Unopened ampoules should be stored in the dark at or below -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.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. The contents of the ampoule should be dissolved in 1 ml of distilled water and the resulting solution stored on ice and used within 4 hours.

8. STABILITY

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

NIBSC follows the policy of WHO with respect to its reference materials.

Recent accelerated degradation studies on samples held at elevated temperatures for 13.3 years suggest good long term stability of 01/580 and predict a loss of activity of 0.009% per year for ampoules stored at -20°C. The product is stable for short periods at higher temperatures and is routinely shipped under ambient conditions.

9. REFERENCES

Whitton C. Sands D. Lee T. Chong A. Longstaff C.



A reunification of the USC ("NIH") and International Unit into a single standard for Thrombin.
Thromb Haemost. 2005 Feb; 93(2) : 261-6.

10. ACKNOWLEDGEMENTS

The organisers of the study gratefully acknowledge the help and materials provided by the manufacturers of the thrombin preparations included in this study, Baxter Bioscience, Vienna, Austria, and Bio Products Laboratory, Elstree, Herts UK, and all the study participants who took part in the international collaborative study.

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):	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.

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: 10mg
Toxicity Statement: Toxicity not assessed
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_efstandardsrev2004.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.