Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
WHO 3rd International Standard for Thrombin
NIBSC code: 19/188
Instructions for use
(Version 1.0, Dated 24/11/2020)

1. INTENDED USE

The above named Standard has been developed to replace the 2nd International Standard for Thrombin (01/580). The potency of the new Standard is 90 IU/ampoule.

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

The potency was assigned to this Standard through an international collaborative study comprising 20 laboratories in 13 countries. Participants were requested to perform their in-house methods for thrombin potency determinations. These were performed with fibrinogen or plasma substrates or chromogenic methods. The potency of the candidate materials was calculated relative to the 2nd International Standard for Thrombin (01/580) using parallel line analysis. The conclusion of the study was candidate material 19/188 was a suitable replacement standard for 01/580 with a potency of 90 IU/ampoule. This was based on results from fibrinogen and plasma clotting assays. Results from chromogenic assays were not used in the calculation of this value. The Standard was established by the Expert Committee on Biological Standardisation of the WHO in August 2020.

4. CONTENTS

Country of origin of biological material: United Kingdom.
The thrombin used to make the Standard was generously provided by a manufacturer as a purified solution of human alpha-thrombin prepared from pooled plasma. The material was shipped to NIBSC as a frozen solution where it was then thawed and diluted to a concentration of approximately 90 IU/ml in a solution of 10 mM Hepes, pH 7.4, containing 0.15 M NaCl and 5 mg/ml human albumin solution.

5 mL DIN ampoules were filled with 1 mL aliquots of the diluted material and lyophilised following NIBSC procedures. A total of 9720 ampoules of 19/188 were available for use. Precision of the fill was monitored by check-weights evenly spaced throughout the total fill. The results are expressed as the % coefficient of variation (cv), where n is the number of ampoules sampled to determine each parameter: mean filling weight = 1.0070 g (cv = 0.14 %, n = 343); mean dry weight = 0.01643 g (cv = 1.16 %, n = 6); mean residual moisture content = 0.49 % (cv = 27.68 %, n = 12); mean oxygen headspace = 0.43 % (cv = 33.92 %, n = 12).

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 2nd International Standard for Thrombin (01/580) (1), which in turn was very similar to the 1st International Standard for Alpha Thrombin (89/588) (2).

5. STORAGE

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

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
To reconstitute, allow the ampoule to warm to room temperature and ensure that the lyophilised material is all in the base of the ampoule before carefully snapping off the top of the ampoule. The contents should be reconstituted using 1 mL of distilled water and mixed gently to produce a clear, colourless solution. This solution should be stored on ice and used as soon as possible by dilution into appropriate assay buffer under conditions defined for your assay. Following reconstitution, the activity is stable for several hours when the solution is maintained on ice. However, the potency is not guaranteed after further freezing and thawing of the reconstituted solution.

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. It is the policy of WHO not to assign an expiry date to their international reference materials and they remain valid with the assigned potency until withdrawn or amended. Predictions on long term stability are made by monitoring ampoules stored under accelerated degradation conditions over time.

9. REFERENCES

(1) Whitton C, Sands D, Lee T, Chang A, Longstaff C. A reunification of the US ("NIH") and International Unit into a single standard for Thrombin. Thromb Haemost. 2005;93(2):261-6.
(2) Gaffney PJ, Heath AB, Fenton JW, 2nd. A collaborative study to establish an international standard for alpha-thrombin. Thromb Haemost. 1992;67(4):424-7.

A report of the collaborative study to calibrate the standard is available from WHO, reference WHO/BS/2020.2384.

10. ACKNOWLEDGEMENTS

We are grateful to all the participants that took part in the collaborative study, and to the FXIII and Fibrinogen Subcommittee of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH).

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

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Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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: 10 mg
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_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.

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