WHO International Standard
3rd INTERNATIONAL STANDARD FOR PLASMIN
NIBSC code: 97/536
Instructions for use
(Version 3.0, Dated 26/03/2008)

1. INTENDED USE
The 3rd International Standard for plasmin, consists of ampoules (coded 97/536) containing the residue after freeze-drying of a solution of a highly purified preparation of human plasmin admixed with human albumin, lactose and buffer salts. This preparation was established by the Expert Committee on Biological Standardisation of the World Health Organisation in October 1998.

The 3rd IS for plasmin (97/536) was calibrated in terms of the 2nd IRP for plasmin (77/588) which had been calibrated in a previous study to contain 10 IU of plasmin activity per ampoule (1). The calibration of the 3rd IS for plasmin was performed using a chromogenic assay (2) in an international collaborative study involving 6 laboratories. The potency was assigned as the geometric mean of all valid assays in all laboratories, giving a value of 5.3 IU per 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
5.3 IU per ampoule

4. CONTENTS
Country of origin of biological material: United Kingdom.

For reconstitution allow ampoules to warm to room temperature. Open ampoule, taking care to ensure that all material is in the lower part, and reconstitute with 1.0ml of water. Since glycerol has been used for many years to stabilize plasmin some workers may wish to use their assay buffer containing 25% glycerol.

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.

Material for the proposed 3rd IS for plasmin was supplied in a single container and in a frozen state. The material was supplied by Behring Diagnostics GmbH, Marburg, Germany and was prepared from affinity-purified plasma plasminogen by activation with urokinase immobilized on Sepharose beads. The final solution from which the freeze-dried contents of each ampoule were derived was adjusted to contain about 5 IU/ml plasmin, 5 mg/ml human albumin and 5 mg/ml lactose. The buffer solution was 0.1 M phosphate (pH=7.4) containing 0.01 M l-lysine. The ampouling, freeze-drying and lyophilisation were performed at NIBSC under conditions used for international biological standards as described previously (4).

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.

A physiological assay using fibrin in a clot lysis assay was used at NIBSC to assess the loss of potency in degradation samples stored at elevated temperatures for 38 weeks, as well as the chromogenic procedure. The clot lysis assay was specifically used because of earlier observations that loss of enzymatic activity due to heat degradation is best measured using a natural substrate such as fibrin (3).

The clot lysis assays appear to detect some degradation at +37°C and +45°C. For the clot lysis assays, the long term stability of the proposed International Standard for plasmin was predicted using the Arrhenius equation. The predicted losses are 0.79% per year at the storage temperature of -20°C and 1.94% per month at 37°C. Since the assay in routine use is the chromogenic assay the degradation from such assays was also used to assess stability for 38 weeks. Thus it was concluded that the proposed standard has a suitable stability profile to serve as an International Standard.

9. REFERENCES

10. ACKNOWLEDGEMENTS
Are made to the suppliers of the material used for the standard and to all the participants in the 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:
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
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<tbody>
<tr>
<td>Physical appearance: Solid</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable:</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material of human origin</td>
</tr>
</tbody>
</table>

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 |
| 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_refstandardsrev2004.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.