WHO International Standard

WHO 2nd INTERNATIONAL STANDARD FOR PROTEIN S, PLASMA, HUMAN
NIBSC code: 03/228
Instructions for use
(Version 3.0, Dated 10/02/2014)

1. INTENDED USE
The 2nd International Standard for Protein S, Plasma, Human, consists of ampoules (code-labelled 03/228) containing 1 ml aliquots of pooled fresh human plasma, freeze-dried. This standard has been assigned potencies for total and free Protein S antigen and for Protein S function.

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 2nd International Standard was calibrated in an international collaborative study involving 20 laboratories for Total Protein S antigen, Free Protein S antigen and Protein S function by assay against the 1st International Standard (93/590). The standard was established by the WHO Expert Committee on Biological Standardisation in October 2006. Details of the collaborative study are available in the WHO document WHO/BS/06.2046. The following values have been assigned to the 2nd International Standard:

- Total Protein S antigen: 0.83 IU per ampoule
- Free Protein S antigen: 0.81 IU per ampoule
- Protein S function: 0.77 IU per ampoule

Uncertainty: the International Unit of 03/228 may be considered to be the coefficient of variation of the ampoule filling, which was determined to be 0.09 %.

4. CONTENTS
Country of origin of biological material: United Kingdom.
The 2nd International Standard was prepared in February 2004 from a plasma pool collected from 24 normal healthy donors. Blood was collected into CPD-adenine anticoagulant in plastic packs, at a ratio of 450 ml blood to 63 ml anticoagulant. Each donation underwent leuko-filtration and centrifugation before storage at -70 °C. The units were thawed in a waterbath at 37°C and pooled on the day of filling. The final pool was buffered by the addition of HEPES (N-[2-Hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]) to a final concentration of 39 mmol/L and Glycine to a final concentration of 0.96 % w/v.

DISTRIBUTION INTO AMPOULES
The pooled plasma was kept at 4°C throughout distribution into approximately 5,500 ampoules, then freeze-dried and secondary desiccated according to the requirements for International Biological Standards (1). The coefficient of variation for the liquid fill was 0.09 % and the mean fill weight was 1.0063 g (range 1.0042 - 1.0086 g). The final C of freeze-dried material has a mean dry weight of 0.093 g and mean residual moisture of 0.064 %.

5. STORAGE
Unopened ampoules should be stored at -20°C or below. 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.

Reconstitute the total contents of each ampoule of the Standard with 1.0ml of distilled water, using gentle shaking. Transfer the reconstitutes Standard to a plastic tube and keep on melting ice. Under these conditions the standard has been found to be sufficiently stable to be used over a 4 hour period. Storage of the reconstituted Standard under different conditions must be validated locally by users.

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 expiry dates to international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Accelerated degradation studies, which involve potency estimation of ampoules stored at elevated temperatures relative to ampoules stored at below -150°C, have shown that the material is very stable in unopened ampoules stored at -20°C. Predicted loss over one year whilst stored at -20°C is less than 0.1% for all measured parameters. These studies have shown that when stored at -20°C or below the assigned values will remain valid until the material is withdrawn or replaced.

Once reconstituted the activity of this preparation is assured for three hours when stored on melting ice.

9. REFERENCES
10. ACKNOWLEDGEMENTS
The efforts of the participants in the collaborative study, the members of the Plasma Coagulation Inhibitors sub-committee and the staff of the Centre for Biological Reference Materials (NIBSC) are gratefully acknowledged.

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/jcltm/
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Corrosive: No</th>
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<tbody>
<tr>
<td>Physical appearance: Solid</td>
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<tr>
<td>Stable: Yes</td>
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<td>Hygroscopic: Yes</td>
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<tr>
<td>Flammable: No</td>
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<tr>
<td>Other (specify): Contains material of human origin</td>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
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<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
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<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
<td></td>
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<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
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</tbody>
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<tr>
<th>Suggested First Aid</th>
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<tr>
<td>Inhalation: Seek medical advice</td>
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<tr>
<td>Ingestion: Seek medical advice</td>
<td></td>
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<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
<td></td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
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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: ~90mg
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.