WHO International Standard
1st International Standard for Unfractionated Heparin for Molecular Weight Calibration
NIBSC code: 07/324
Instructions for use
(Version2.0, Dated 12/10/2026)



1. INTENDED USE

The 1st International Standard (IS) for Unfractionated Heparin for Molecular Weight Calibration consists of ampoules, coded 07/324, containing aliquots of a freeze-dried material prepared from porcine mucosa. This preparation was established as the 1st IS by the Expert Committee on Biological Standardisation (ECBS) in April 2026 – see WHO/BS/2026.2500. The material is intended for use as a broad standard calibrant to measure the weight average molecular weight of unfractionated heparin using site exclusion / gel permeation chromatography systems with detection by refractive index (1).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.
The material is not of human or bovine origin. 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

There is no unitage assigned to this material. The standard has a broad standard table (BST) associated with it which is shown in Table 1 and was qualified in an International Collaborative Study involving 12 laboratories from 7 countries. Please see ECBS report for more details.

4. CONTENTS

Country of origin of biological material: United Kingdom.
In 2008, 251.6 g bulk material was dissolved in 25 litres water for injection. The solution was distributed into 25,000 ampoules (CV for volume of fill 0.12%, n = 970), coded 07/324. The contents of the ampoules were then freeze-dried under conditions recommended for international standards (2,3). The mean dry weight of the freeze-dried plug was 10.3 mg, with a water content of 0.60%.

5. STORAGE

Unopened ampoules should be stored in the dark at or below 20°C. Please note because of the inherent stability of lyophilised material, NIBSC may ship these materials at ambient temerature.

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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. The calibrant is intended for use in the determination of molecular weight distribution of unfractionated heparin by size exclusion chromatography (SEC) also sometimes known as gel permeation chromatography (GPC), using refractive index (RI) detection. Once the ampoule has been brought to ambient temperature, the contents should be reconstituted with an appropriate buffer for use in a SEC / GPC system. Typically, 2 ml buffer can be used for a 5 mg/ ml solution that can be injected for a chromatography run, due to the nature of RI a precise concentration is not necessary.

Reconstituted material can be employed to calibrate a chromatography system by broad standard calibration using the molecular weight distribution information listed in Table 1. For each molecular weight (M) in the Table, the percent of the sample above M (%M) and the percent below M are given. The use of specialist SEC computer sofeware for calibration of the chromatography system and for calculation of the molecular weights of unfractionated heparin samples is strongly recommended. Alternative calculation using a spreadsheet can be found in reference 1.

This material is not suitable for use to calibrate a system intended to measure the molecular weight of low molecular weight heparin – please use 05/112 for this purpose.

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 the WHO with repect to its reference materials. It is the policy of the WHO not to assign an expiry data to their international reference materials, and as such they remain valid with the assigned value until withdrawn or amended.

Stability studies have indicated that the material is inhertently stable when stored at -20°C, and suffers no loss in performance following a short period at ambient temperature.

9. REFERENCES

1 – Chromatographic molecular weight measurements for heparin, its fragments and fractions, and other glycosaminoglycans. Mulloy B, Hogwood J. Methods Mol Biol. 2015; 1229:105-18.
2 – Campbell PJ. International biological standards and reference

preparations.1. Preparation and presentation of materials to serve as standards and reference preparations. J Biol Standardisdation 1974: 2: 249-267.
3 – Recommendations for the Preparation, characterisation and establishment of international and other biological reference standards (revised 2004). In: WHO TRS, No 932, 2006, Annex 2 pp. 114-119 (section A.7)

10. ACKNOWLEDGEMENTS

All the participants in the international collaborative study and the manufacturers for their kind donation of heparin samples for this study: Aspen NDB, Biolberica SAU and Opocrin SpA

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):	
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: 10.3 mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** False

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 https://www.who.int/publications/m/item/annex2-trs932(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.

Table 1: Broad Standard Table for 07/324

Point	M (Da)	% below M	% above M
1	6000	3.2	96.8
2	8000	10.4	89.6
3	10000	19.8	80.2

4	12000	31.7	68.3
5	14000	43.4	56.6
6	16000	55.5	44.5
7	18000	66.0	34.0
8	20000	74.4	25.6
9	22000	80.3	19.7

10	24000	84.4	15.6
11	26000	87.5	12.5
12	28000	90.1	9.9
13	32000	93.4	6.6
14	36000	95.6	4.4
15	40000	97.0	3.0