



**WHO International Standard  
2nd International Standard Low Molecular Weight Heparin for  
Molecular Weight Calibration  
NIBSC code: 05/112  
Instructions for use  
(Version 4.0, Dated 27/11/2013)**

### 1. INTENDED USE

The 2nd International Standard Low Molecular Weight Heparin for Molecular Weight Calibration consists of ampoules, coded 05/112, containing aliquots of a freeze-dried material prepared from porcine mucosa. This preparation was established as the 2nd International Standard Low Molecular Weight Heparin for Molecular Weight Calibration by the Expert Committee on Biological Standardisation of the World Health Organisation in 2007

### 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 assigned unitage associated with this standard. The standard was calibrated by 15 laboratories in 10 countries, against the 1st International Reference Reagent Low Molecular Weight Heparin for Molecular Weight Calibration (1). It is characterised by the Table in Appendix 1.

### 4. CONTENTS

Country of origin of biological material: Denmark.  
In June 2005, 251.3 mg bulk material was dissolved in 10 litres water for injection. The solution was distributed at 4°C into 10000 ampoules (CV for volume of fill 0.15% (n=136)), coded 05/112. The contents of the ampoules were then freeze-dried under the conditions normally used for international biological standards. The mean dry weight (n=6) of the freeze-dried plug was 23.5 mg, with a water content of 0.29%.

### 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. 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 the molecular weight distribution of low molecular weight heparins by size exclusion chromatography (SEC, also sometimes known as gel permeation chromatography (GPC)). It may be used to calibrate a chromatography system by broad standard calibration (as has been described for the previous calibrant (2)), using the molecular weight distribution information as listed in the table in Appendix 1. For each molecular weight (M) in the

Table, the percent of sample above M (%>M) and the percent of sample below M (%<M) are given. The use of specialised SEC computer software for calibration of the chromatography system and for calculation of the molecular weights of low molecular weight heparin samples is strongly recommended. It should be noted that the 2nd International Standard Low Molecular Weight Heparin for Molecular Weight Calibration is not suitable for use in the method of Nielsen (3).

### 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 the WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Accelerated degradation studies, which involves potency estimation of ampoules stored at elevated temperatures relative to ampoules stored on liquid nitrogen vapour (approx. -150°C) for this preparation have, to date shown no loss in activity. The accelerated degradation study and real time monitoring will continue for the lifetime of the standard.

### 9. REFERENCES

1. Mulloy B, Heath A, Behr-Gross ME (2007) *Pharmeuropa Bio* 2007(1): 29-48
2. Mulloy B, Gee C, Wheeler SF, Wait R, Gray E, Barrowcliffe TW (1997) *Thrombosis and Haemostasis* 77, 668-674
3. Nielsen JI, (1992) *Thrombosis and Haemostasis* 68, 478-480

### 10. ACKNOWLEDGEMENTS

Acknowledgements are made to the joint organisers of the collaborative study at the European Directorate for the Quality of Medicines, in particular to the study co-ordinator Dr M.-E. Behr-Gross, as well as to the participants in the study. We also thank the donors of the bulk material for this standard, Leo Pharma A/S, Industriparken 55, DK-2750 Ballerup, Denmark.

### 11. FURTHER INFORMATION

Further information can be obtained as follows;  
This material: [enquiries@nibsc.org](mailto: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](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](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](mailto: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.	

## 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](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.

### APPENDIX 1: BROAD STANDARD TABLE FOR 05/112

Point	Log <sub>10</sub> (M)	M	% >M	% <M
1	2.78	600	0.40	99.60
2	3.08	1200	3.87	96.13
3	3.26	1800	8.94	91.06
4	3.38	2400	14.49	85.51
5	3.48	3000	20.68	79.32
6	3.56	3600	27.20	72.80
7	3.62	4200	33.89	66.11
8	3.68	4800	40.49	59.51
9	3.73	5400	46.83	53.17
10	3.78	6000	52.92	47.08
11	3.82	6600	58.59	41.41
12	3.86	7200	63.89	36.11
13	3.92	8400	72.96	27.04
14	3.98	9600	80.09	19.91
15	4.08	12000	89.21	10.79
16	4.13	13600	92.96	7.04
17	4.19	15600	95.95	4.05
18	4.26	18000	97.77	2.23

## 15. LIABILITY AND LOSS

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## 16. INFORMATION FOR CUSTOMS USE ONLY

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 23.5 mg
<b>Toxicity Statement:</b> Toxicity not assessed
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> No