



**WHO Reference Reagent
Insulin-Like Growth Factor-II (Human, Recombinant)
NIBSC code: 96/538
Instructions for use
(Version 4.0, Dated 18/01/2008)**

1. INTENDED USE

This consists of a batch of ampoules (coded 96/538) containing *E. coli* cell-derived insulin-like growth factor-II (IGF-II) analysed by international collaborative study and established as the First WHO Reference Reagent for Insulin-like Growth Factor-II by the Expert Committee on Biological Standardization of the World Health Organisation (1,2).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

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 preparation in ampoules coded 96/538 is assigned a potency of 5000 International Units (IU) per ampoule. On the basis of the immunoassay results, preparation 96/538 is assigned a nominal mass content of 5µg per ampoule. The bioactivity units and the immunoactivity units should not be assumed to be interconvertible.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1ml of a solution that contained:

4.5mg NaCl	3.0mg arginine
4.0mg Na phosphate	0.1mg Tween 20
30.0mg trehalose	rec IGF-II

5. STORAGE

The ampouled preparation 96/538 appears to be sufficiently stable to serve as a WHO Reference Reagent since there was little evidence of any significant loss of activity even after storage at +45°C for 8 months.

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

For practical purposes each ampoule contains the same quantity of IGF-II. The entire content of each ampoule should be completely dissolved in an

accurately measured amount of buffer solution. No attempt should be made to weigh out portions of the freeze dried powder. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

8. PREPARATION OF AMPOULES

8.1 Bulk material

Highly purified rDNA-derived IGF-II, expressed in *E. coli* (3), was kindly donated to WHO by Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.

8.2 Distribution into ampoules

The preparation was received as a lyophilised white powder (nominal 5.15mg) which was dissolved in 2.575ml 0.0035M HCl to a concentration of 2.0mg/ml. After dilution (2.5ml up to 1L) with a solution containing 0.45% (w/v) sodium chloride, 0.4% (w/v) sodium phosphate, 3.0% (w/v) trehalose, 0.3% (w/v) arginine and 0.01% Tween 20, pH 6.99, the preparation was distributed into ampoules as 1ml aliquots. The ampouled preparation was lyophilised and sealed according to procedures described by WHO for International Biological Standards (4) and stored at -20°C in the dark.

9. COLLABORATIVE STUDY

The preparation in ampoules coded 96/538 was evaluated by international collaborative study in which eight laboratories in four countries took part. Assays contributed included in vitro assays based upon proliferation in various cell lines and immunoassays. The study was designed to:-

- compare, by bioassay and immunoassay, the ampouled preparation of recombinant IGF-II with local standards presently in use.
- calibrate the preparation of IGF-II for use as a reference reagent.
- assess the stability of the proposed reference reagent after accelerated thermal degradation.

9.1 Activity of ampoule contents

Estimates by assays based on biological function (bioassay and competitive protein binding assay) and immunoassay (RIA, ELISA and IRMA) were similar and consistent with local standards, assuming an ampoule content of 5µg. Accordingly, the preparation coded 96/538 was established as the First WHO Reference Reagent for IGF-II, human, recombinant, with a defined ampoule content of 5000 IU per ampoule. On the basis of the immunoassay results, preparation 96/538 was assigned a nominal mass content of 5µg per ampoule.

9.2 Stability

The ampouled preparation 96/538 appears to be sufficiently stable to serve as a WHO Reference Reagent since there was little evidence of any significant loss of activity even after storage at +45°C for 8 months.



10. STABILITY

It is the policy of 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.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact standards@nibsc.ac.uk

In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

11. REFERENCES

1. WHO Technical Report Series No. 904, 2002; 25
2. Rafferty B, Rigsby P & Gaines Das RE. Multicentre collaborative study to calibrate IGF-II by bioassay and immunoassay: establishment of the First WHO Reference Reagent. *Growth Hormone & IGF Research* 2001; 11: 18-23.
3. Furman TC, Epp J, Hsiung HM, et al. Recombinant human insulin-like growth factor II expressed in *Escherichia coli*. *Biotechnology* 1987; 5: 1047-1051.
4. WHO Technical Report Series No.800, 1990; 181-214

12. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants, Lilly Research Laboratories who kindly donated the recombinant IGF-II and Dr P Dawson and Standards Division for preparation of the ampouled materials.

13. 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

14. 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

15. 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.

16. 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 lyophilised powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Can react with oxidising materials. Avoid contact with acids and alkalis	
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. 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.

18. 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: 42mg
Toxicity Statement: Non-toxic
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_biologicalstandardsrev2004.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.