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
4.0mg Na phosphate
3.0mg arginine
30.0mg trehalose
0.1mg Tween 20
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
month.
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

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 aliquotted, 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

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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th>Corrosive: No</th>
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<tbody>
<tr>
<td>Stable:</td>
<td>Oxidising: No</td>
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<tr>
<td>Hygroscopic:</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable:</td>
<td>Handling: See caution, Section 2</td>
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</tbody>
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Other (specify): Can react with oxidising materials. Avoid contact with acids and alkalies

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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
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</thead>
<tbody>
<tr>
<td>* 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.</td>
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<table>
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<tr>
<th>Net weight:</th>
<th>42mg</th>
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<table>
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<th>Toxicity Statement:</th>
<th>Non-toxic</th>
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<tr>
<th>Veterinary certificate or other statement if applicable.</th>
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<tr>
<td>Attached: No</td>
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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_biolefstandardsrev2004.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.