WHO International Standard
ENDOTOXIN (2nd International Standard)
NIBSC code: 94/580
Instructions for use
(Version 4.0, Dated 18/07/2013)

1. INTENDED USE
Calibrant for bacterial endotoxins test

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
10 000 IU/vial = 10 000 EU/vial

4. CONTENTS
Country of origin of biological material: USA.
Each vial contains the residue after freeze-drying of 1.0 ml of a solution
that contained:
E. coli 0113: H10: K - endotoxin 1 µg
Lactose 10 mg
Polyethylene glycol 1 mg

5. STORAGE
Store unopened vials at -20°C or below in the dark.
Please note: because of the inherent stability of lyophilized
material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
Vials have a ‘flip-up’ circular cap. Either on the cap or the collar of the
vial, there is an indication of the point at which to lever off the cap. This
exposes an area of the stopper through which reconstitution and
withdrawal of the preparation can be made using a hypodermic needle
and syringe. If use of a pipette is preferred, then fully remove the metal
collar using, for example, forceps, taking care to avoid cuts by wearing
appropriate gloves. Remove the stopper for access. Care should be
taken to prevent loss of the contents.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried
material prior to reconstitution.
Add 5 ml of sterile, pyrogen free water (LAL Reagent Water) to the vial.
Reconstitute by mixing intermittently for a total time of not less than 30
min, using a vortex mixer. The resulting solution of 2000 IU (= EU) per ml
is to be used as a stock solution for preparing serial dilutions. The stock
solution may be divided into aliquots immediately after reconstitution and
the aliquots stored frozen at -40°C or below. Alternatively, the stock
solution may be stored at +2-8°C for not more than 14 days. Aliquots of
the stock solution are to be taken (for preparing serial dilutions) after
vigorous mixing for at least 5 minutes. Subsequent dilutions are to be
mixed for not less than 30 seconds.

Suitable precautions should be taken in the use and disposal of the vial
and its contents.

8. STABILITY
NIBSC follows the policy of WHO with respect to its reference materials.
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. 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. Users who have data supporting any
deterioration in the characteristics of any reference preparation are
encouraged to contact NIBSC.

9. REFERENCES
standard for endotoxin: calibration in an international collaborative study.

10. ACKNOWLEDGEMENTS
The endotoxin contained in this ampoule was generously contributed by the
FDA/USP to WHO.

11. FURTHER INFORMATION
Further information can be obtained as follows;
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
referred to, 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

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>Yes</td>
</tr>
<tr>
<td>Hygroscopic</td>
<td>Yes</td>
</tr>
<tr>
<td>Flammable</td>
<td>No</td>
</tr>
<tr>
<td>Corrosive</td>
<td>No</td>
</tr>
<tr>
<td>Oxidising</td>
<td>No</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
<td></td>
</tr>
<tr>
<td>Contains material of bacterial origin</td>
<td></td>
</tr>
</tbody>
</table>

Toxicological properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
<td>Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
<td>Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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<table>
<thead>
<tr>
<th>Suggested First Aid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Action on Spillage and Method of Disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spillage of ampoule contents should be taken up with wetted absorbent material. Rinse area with an appropriate cleaning agent followed by water. Absorbent materials used to treat spillage should be treated as biological waste.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
</tr>
</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>
</tr>
<tr>
<td>Net weight: 11mg</td>
</tr>
<tr>
<td>Toxicity Statement: Toxicity not assessed</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable.</td>
</tr>
<tr>
<td>Attached: No</td>
</tr>
</tbody>
</table>

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_bi ologicalstandardsrev2004.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.