WHO International Standard
3rd WHO International Standard for Erythropoietin, recombinant,
for bioassay
NIBSC code: 11/170
Instructions for use
(Version 1.0, Dated 30/10/2012)

1. INTENDED USE
The second International Standard (IS) for Erythropoietin (EPO) in
ampoules coded 88/574 has been widely used for the calibration of
preparations of recombinant DNA-derived EPO by bioassay. Stocks of the
2nd IS are exhausted and the World Health Organization (WHO) Expert
Committee on Biological Standardization (ECBS) has recognized (2010)
the need for a replacement International Standard for EPO for the
assignment of potency to therapeutic preparations of recombinant human
EPO used in the treatment of anaemia.

A new preparation of recombinant EPO has been filled into ampoules
(NIBSC Code 11/170) and has been characterized by in vivo bioassay in an
international collaborative study with expert laboratories and was
established as the 3rd International Standard at the 63rd meeting of the
ECBS. This material replaces the 2nd IS.

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

The preparation contains material of human origin, and either the final
product or the source materials, from which it is derived, have been
tested and found negative for HBsAg, anti-HIV and HCV RNA. 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
Each ampoule contains 1650 IU of EPO

4. CONTENTS
Country of origin of biological material: USA.
Each ampoule contains the residue after freeze-drying of 0.5 ml of a
solution that contained:

- Recombinant human EPO: approximately 11µg
- Human serum albumin: 0.2% (w/v)
- Trehalose: 1.0% (w/v)
- NaCl: 0.12% (w/v)

5. STORAGE
Unopened ampoules should be stored at -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.
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
No attempt should be made to weigh out any portion of the freeze-dried
material prior to reconstitution.

For practical purposes, each ampoule contains the same quantity of
recombinant human EPO. The entire content of each ampoule should be
completely dissolved in an accurately measured amount of buffer solution.
The use of water to reconstitute ampoule contents is not recommended. The
material has not been sterilized and the ampoules contain no bacteriostat.

COLLABORATIVE STUDY
The preparation was evaluated in a collaborative study in which fifteen
laboratories in seven countries took part, organized with the following aims:

1) To calibrate the candidate preparation, 11/170 relative to the 2nd IS
   (88/574) for EPO by in vivo bioassays.

2) To determine the stability of the candidate preparation 11/170 by
   comparison with ampoules stored at elevated temperatures as part of an
   accelerated degradation stability study.

The geometric mean potency for the candidate standard was 1648 IU per
ampoule (n=15; 95% confidence limits 1562 - 1738; GCV 10.1%)

The candidate preparation 11/170 is sufficiently stable to serve as an
International Standard. Analysis of the thermally accelerated degradation
samples in this study demonstrated no detectable loss of potency at these
elevated temperatures. This suggests that 11/170 is likely to be highly stable
under long term storage at -20°C.

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.

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.

In addition, once reconstituted, diluted or aliquoted, users should
check 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.

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

9. REFERENCES
Further information can be obtained from the report of the collaborative study
which is available from the WHO website: http://www.who.int/biologicals/en/

10. ACKNOWLEDGEMENTS
We gratefully acknowledge the important contributions of all the participants
and the manufacturer of the therapeutic EPO for the kind donation of
material.

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:
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
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<tbody>
<tr>
<td>Physical appearance: Freeze dried powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
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<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains material of human origin</td>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
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<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>
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</tbody>
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<table>
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<tr>
<th>Suggested First Aid</th>
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<tbody>
<tr>
<td>Inhalation:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Ingestion:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes:</td>
<td>Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin:</td>
<td>Wash thoroughly with water.</td>
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<tr>
<th>Action on Spillage and Method of Disposal</th>
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<tr>
<td>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.</td>
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15. LIABILITY AND LOSS
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