Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Working Standard
Erythropoietin, human, recDNA
NIBSC code: 87/690
Instructions for use
(Version 1.01, Dated 20/06/2011)

This material is not for in vitro diagnostic use.

1. INTENDED USE

A significant increase in the demand for the 2nd International Standard, coded 88/574 has meant that stocks are now exhausted and the replacement preparation, the 3rd WHO International Standard, will not be available until the end of 2012. To ensure the continued availability of a suitable reference material, calibrated in IU and traceable to the 2nd IS, we are making available an interim standard, coded 87/690. This standard was assigned a potency in the same collaborative study as 88/574 (described in Storring PL & Gaines Das RE 1992. J. Endocrinol. 134:459-484) and was also deemed to be suitable as an International Standard for the calibration of therapeutic preparations of EPO by bioassay. It is important to note that the potency assigned to 87/690 is 81IU which is some 50% less than the potency assigned to the 2nd IS, 88/574. This means that users will require additional ampoules for their calibration purposes (i.e. 3 ampoules of 87/690 will be required for every 2 ampoules of 88/574).

The interim standard can be ordered from the NIBSC website (www.nibsc.ac.uk) in the usual manner.

2. CAUTION

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

The preparation contains albumin of human origin which has been tested and found negative for HBsAG and HIV antibody. The preparation has subsequently been tested and found negative for anti-HCV and HCV RNA by PCR. 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 81 INTERNATIONAL UNITS of erythropoietin, recombinant (by definition).

4. CONTENTS

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

rEPO	approx	0.918 µg
Trehalose	approx	5 mg
Human plasma albumin	approx	1 mg
Sodium chloride	approx	0.6 mg

Nitrogen gas at slightly less than atmospheric pressure.

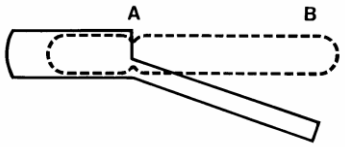
5. STORAGE

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

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

For practical purposes each ampoule contains the same amount of the same materials. Dissolve all the contents in a known amount of buffer solution. No attempt should be made to weigh portions of the freeze-dried powder.
For economy of use the solution can be kept for several months if an anti-bacterial preservative is added and the solution is subdivided into several small containers, which are frozen rapidly to below –70 ºC and then stored below –30 ºC in the dark; repeated freezing and thawing should be avoided. If extensive dilutions are prepared, a carrier protein (0.1% w/v) should be added, which is free of peptidase.
The material has not been sterilized and contains no bacteriostat.

7.1. PREPARATION OF AMPOULES

Four preparations of rEPO, stated by their manufacturers to be highly purified, were generously donated to WHO as candidate ISs by: Amgen, Genetics Institute through the good offices of Boehringer Mannheim GmbH, Integrated Genetics Inc in collaboration with Behringwerke AG, and the Snow Brand Milk Products Co Ltd. Methods for the preparation of rEPO and details of its characterization have been published by these manufacturers (Jacobs et al, 1985; Lin et al, 1985; Davis et al, 1987; Recny et al, 1987; Sasaki et al, 1987; Sasaki et al, 1988; Goto et al, 1988; Takeuchi et al, 1988; Tsuda et al, 1988 and by Integrated Genetics Inc in European Patent Publication No EP 0267678). Two of these preparations were synthesized in Chinese hamster ovary cells, another was synthesized in baby hamster kidney cells and the other in the mouse C127 fibroblast cell.

The protein content of these preparations was determined from the absorbance at 280nm of their solutions at ca. pH 7 after correction for the turbidity of the solutions from their absorption spectra between 320 and 360nm (Beaven & Holiday, 1952). The absorbance of a 1% (w/w) solution of EPO at 280nm in a 1cm light-path was assumed to be 8.0.
Each of the four candidate ISs were dispensed into ampoules in the same way. Bulk EPO was dissolved in, or (if obtained as a solution) diluted with a diluent containing 0.2% (w/v) purified human plasma albumin (free of peptidase activity, Lister Institute, Elstree), 1% w/v trehalose and 3mM sodium chloride to give an EPO concentration of between 130 and 350µg/ml. The solution was passed through a (0.45µm) membrane filter (Millex HA, Millipore SA, 67-Molsheim, France) and made up with the diluent to 2200g. The solutions were then distributed into ampoules as approximately 0.5ml aliquots. Solutions of EPO were kept at 4°C throughout. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen (Campbell, 1974; WHO ECBS, 1990). The batch of ampoules coded 87/690 was prepared on 3rd December 1987.

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
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7.2. COLLABORATIVE STUDY

An international collaborative study had been undertaken with four rEPOs, including the rEPOs which were eventually established as the 1st IS (87/684) and the 2nd IS (88/574) and of two other candidate ISs of rEPO (87/690 and 87/696), by 26 laboratories in 11 countries, using a wide variety of in-vivo and in-vitro bioassays and immunoassays (Storring & Gaines Das 1992). The bulk rEPO used to prepare the 1st IS and the 2nd IS had been synthesized in Chinese hamster ovary cell lines, although by different manufacturers, and those used to prepare the other two candidate ISs had been synthesized in baby hamster kidney and mouse C127 fibroblast cell lines, respectively. Ampoules of the 1st IS, the 2nd IS and of the other two candidate ISs were all prepared in the same way (Storring & Gaines Das 1992).

On the basis of the results of the study, the participants in the collaborative study agreed to recommend to the WHO ECBS that the preparation in ampoules coded 87/684 be established as the International Standard for rEPO, and that the other three candidate ISs for rEPO were also suitable to serve as international standards (Storring & Gaines Das 1992). Furthermore, the participants agreed to recommend that the potency assigned to the 1st IS and to the other candidate ISs should be based on their calibration in the collaborative study by in-vivo bioassay in terms of the Second International Reference Preparation of Human Urinary EPO, for Bioassay (Annable *et al.* 1972). In the collaborative study, mean estimates by in-vivo and in-vitro bioassays, and by immunoassays, of the EPO content of ampoules of the interim standard (87/690) kept at +20°C and +37°C for 286 days in terms of those of the interim standard kept at -20° did not differ significantly from that of the material kept at -20°C (Storring & Gaines Das 1992). During 2011, estimates of the EPO activity of ampoules of the interim standard kept at +4°C, and +37°C for 23 years were carried out using the normocythaemic mouse assay. The mean estimates of activity as % of that in ampoules kept at -20°C (with 95% confidence limits) were 99.6 (82.8-120.1)% from two assays of ampoules kept at +4°C, and 105.3 (82.4-135.7)% from one assay of the interim standard kept at +37°C. These data therefore indicated that the interim standard appeared to be adequately stable when stored under normal conditions, at -20°C in the dark.
For further details of the interim standard and of its collaborative study see Storring and Gaines Das (1992).

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

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10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to: the participants in the collaborative assay; and to Amgen, Genetics Institute through the good offices of Boehringer Mannheim GmbH, Integrated Genetics Inc with Behringwerke AG, and the Snow Brand Milk Products Co Ltd for donating the rEPO used in the preparation of the Candidate ISs.

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:
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 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.

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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: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin
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.	

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

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: 7mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

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