



**WHO International Standard  
Erythropoietin, Human recombinant  
NIBSC code: 88/574  
Instructions for use  
(Version 4.0, Dated 22/01/2008)**

## 1. INTENDED USE

This consists of a batch of ampoules coded 88/574 which was established as the Second International Standard for Erythropoietin, Recombinant (2<sup>nd</sup> IS) at the 53<sup>rd</sup> Meeting of the WHO Expert Committee on Biological Standardization (WHO ECBS) in February 2003. The Committee assigned an activity to it of 120 international units per ampoule, based on results obtained by in-vivo bioassay. The Committee noted that the potency assigned may not be appropriate for use in other types of assay. The First International Standard for Recombinant DNA-derived Erythropoietin (1<sup>st</sup> IS; in ampoules coded 87/684) was established by the WHO Expert Committee on Biological Standardization (WHO ECBS) in 1990 (WHO Expert Committee on Biological Standardization 1991). This Standard had been widely used for the calibration of assays to control the quality and potency of recombinant EPO (rEPO) used in the treatment of anaemias associated with a wide range of clinical conditions, and had also been used for the calibration of some immunoassay systems for erythropoietin (EPO) used in clinical diagnosis. When stocks of the 1<sup>st</sup> IS became exhausted, it needed to be replaced.

## 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 120 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	0.943 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.

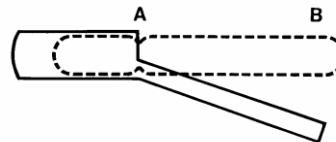
## 5. STORAGE

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 °C and then stored below -30 °C 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.

## 8. 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 87/684 was prepared on 29th October 1987 with one of the two EPO preparations synthesized in Chinese hamster ovary cells. The batch of ampoules coded 88/574 was prepared on 5th October 1988 with the other one of the EPO preparations synthesized in Chinese hamster ovary cells. The batch of ampoules coded 88/574 consisted of 3870 ampoules. The mean weight of filling solution in 79 weighed ampoules of 88/574 was found to be 0.508g with a range as % of mean of 0.886%.

## 9. COLLABORATIVE STUDY

Prior to its establishment by WHO, an international collaborative study had been undertaken of the 1<sup>st</sup> IS and of the 2<sup>nd</sup> IS and of two other candidate ISs of rEPO 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 1<sup>st</sup> IS and the 2<sup>nd</sup> 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 1<sup>st</sup> IS, the 2<sup>nd</sup> 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, including the preparation in ampoules coded 88/574, were also suitable to serve as international standards (Storring & Gaines Das 1992). Furthermore, the participants agreed to recommend that the potency assigned to the 1<sup>st</sup> IS, the 2<sup>nd</sup> 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 in terms of the Second International Reference Preparation of Human Urinary EPO, for Bioassay (Annable *et al.* 1972), namely 120iu/ampoule for the 2<sup>nd</sup> IS. In the collaborative study mean estimates by in-vivo and in-vitro bioassays, and by immunoassays, of the EPO content of ampoules of the 2<sup>nd</sup> IS kept at +20°C and +37°C for 286 days in terms of those of the 2<sup>nd</sup> IS kept at -20°C did not differ significantly from that of the material kept at -20°C (Storring & Gaines Das 1992). During 2002, estimates of the EPO activity of ampoules of the 2<sup>nd</sup> IS kept at +4°C, +20°C and +37°C for 13.7 years were carried out using the normocythaemic mouse assay (2002). The mean estimates of activity as % of that in ampoules kept at -20°C (with 95% confidence limits) were 100 (77.5-130)% from two assays of ampoules kept at +4°C, 102 (84.5-124)% from two assays of ampoules kept at +20°C and 96.7 (76.1-123)% from two assays of the 2<sup>nd</sup> IS kept at +37°C. These data therefore indicated that the 2<sup>nd</sup> IS appeared to be adequately stable when stored under normal conditions, at -20°C in the dark. For further details of the 2<sup>nd</sup> IS 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

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## 11. FURTHER INFORMATION

Further information can be obtained as follows;  
This material: [enquiries@nibsc.org](mailto: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](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](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](mailto: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

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](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

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 7mg
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> 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\\_biol\\_efstandardsrev2004.pdf](http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_efstandardsrev2004.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.