Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
EPIDERMAL GROWTH FACTOR (1-52) Human, rDNA-derived
NIBSC code: 91/550
Instructions for use
(Version 4.0, Dated 02/04/2013)

1. INTENDED USE

The preparation coded 91/550 was established as the 1st International Reference Reagent (IRR) for Epidermal Growth Factor (1-52) by the WHO Expert Committee on Biological Standardization in 1994, following evaluation in an international collaborative study by 12 laboratories. This material is residues 1-52 of the human sequence epidermal growth factor (EGF) synthesized in S. cerevisiae by recombinant DNA technology. EGF (1-52) lacks the carboxyl-terminal arginine of the full length 53-amino acid molecule. The 52- and 53-amino acid forms of EGF were shown to differ in their relative potencies between different assay systems in the WHO international collaborative study of candidate EGF standards.

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

The nominal content of each ampoule is 1.75microgram EGF (1-52). See section 4.

4. CONTENTS

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

EGF (1-52)	3.5 microgram/ml
trehalose	10 mg/ml
sodium phosphate, pH 6.5	10 mmol/l

The nominal content of each ampoule is 1.75microgram EGF (1-52). For a given assay system under defined conditions, the biological activity of EGF (1-52) can be calibrated in terms of the International Standard for EGF (code 91/530, available from NIBSC). However, it should be noted that the dose-response curves for EGF and EGF (1-52) may not be parallel, and caution should be used in expressing the activity of EGF (1-52) in terms of International Units of EGF activity. The relative potencies of EGF and EGF (1-52) may differ between different assay systems.

For most assay systems the relative potencies of EGF and EGF (1-52) may be expected to be within a factor of two, judged on a mass basis. The biological activity of one ampoule of 91/550 should therefore be equivalent to 0.4 to 1.8 ampoules of the IS for EGF (91/530). It is therefore suggested that for an initial comparison, the dilution curves of the EGF (1-52) and EGF should span this concentration ratio.

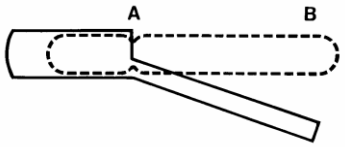
5. STORAGE

The ampoules are shipped at ambient temperature. Unopened ampoules should be stored at -20 degrees C in the dark. For economy of use, it is recommended that the reconstituted solution be subdivided into several small containers and stored at, or below, -40 degrees C. Repeated freezing and thawing should be avoided. The ampoules do not contain bacteriostat and solutions of the ampouled material should not be assumed to be sterile.

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

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

The International Reference Reagent is intended for calibration of local standards. For all practical purposes, each ampoule contains the same quantity of EGF(1-52). The entire contents of each ampoule should be completely dissolved in a known volume of suitable solvent. It is recommended that, when possible, buffer containing carrier protein be used to minimize loss by surface adsorption.

The IRR was evaluated by a range of in vitro bioassays and immunoassays in an international collaborative study. In some assay systems the trehalose may have a small effect, so where high concentrations of EGF (1-52) standard are required, and hence high concentrations of trehalose are present in the assay, it may be advisable to test for any effect of added trehalose, and, if necessary, to calibrate the in-house standard in the presence of trehalose.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities and they should be stored on receipt as indicated on the label. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20ºC or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

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9. REFERENCES

Robinson CJ & Gaines-Das R (1996)
The international standard for epidermal growth factor (EGF): comparison of candidate preparations by in vitro bioassays and immunoassays. Growth Factors 13:163-170

10. ACKNOWLEDGEMENTS

The EGF(1-52) ampouled as this WHO Reference Reagent was selected from preparations generously donated to WHO by Amgen Inc., British Bio-technology Limited, Chiron Corporation and Kabi. Grateful acknowledgements are due also to Wellcome Research Laboratories for the

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

World Health Organization

donation of mouse EGF, and to the participants in the collaborative study in which the candidate standards were evaluated.

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.

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: No	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	
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: 10mg
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable**.** **Attached:** 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_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.