Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
1st International Standard for TNF receptor II Fc fusion protein (Etanercept, Human rDNA derived)
NIBSC code: 13/204
Instructions for use
(Version 1.0, Dated 13/01/2016)

1. INTENDED USE

The World Health Organization (WHO) Expert Committee on Biological standardization (ECBS) recognised the need for a reference standard to control biological assays used in the evaluation of TNF receptor II Fc fusion protein (etanercept). The preparation 13/204 was evaluated in an international collaborative study (described in section 3), and formally adopted by ECBS as the 1st WHO International Standard for in vitro biological activity of etanercept.

The standard can serve to control the performance of biological assays for etanercept and to support the establishment of in-house bioassay standards.

It should be noted that the bioassay unitage is not intended for describing the labelling or dosage of etanercept.

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

The preparation has been assigned a unitage of 10,000 IU of biological activity per ampoule. This unit does not consider the inhibitory activity of the protein against Tumor necrosis factor-alpha (TNF-alpha) and is independent of the amount of TNF-alpha used in various assay systems.

Since the protein is an inhibitor of TNF-alpha, the inhibitory activity of the standard has been established in a cytotoxicity assay using the L929 (murine fibroblast) cell line. Based on ED50 responses from data of nine laboratories, 2.4 IU of this reference standard inhibits the cytotoxic effect of 10-20 IU of 3rd IS for TNF-alpha (coded 12/154) in L929 cytotoxicity assays.

As the inhibitory activity may vary according to the assay format, users should establish a relationship between the units assigned to the WHO international standard, and the unitage assigned to the in-house standard in the assay system being used. Users should also note that the biological activity of TNF-alpha is also likely to vary between different suppliers and this should be controlled by use of an appropriate standard (e.g. WHO IS)

The preparation was tested in a collaborative study involving 28 laboratories in 15 countries, Participants were asked to assay the candidate preparation using assay systems established in house, and to report results. Data were returned for cytotoxicity, apoptosis, reporter gene and binding assays. (see reference in section 9, WHO/BS/2015.2257)

4. CONTENTS

Country of origin of biological material: United Kingdom.

Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution containing:

Etanercept, approximately 5 micrograms
10mM Tris-HCL
1% Sucrose
4% Mannitol
0.2% Human Serum Albumin

(Note: the quantity of etanercept is an approximate figure given for guidance. It is not a formally assigned content and should not be used to calculate or infer a specific biological activity. It also does not provide information on specific activity for marketing authorisation purposes).

The Etanercept protein was expressed in CHO cells.

5. STORAGE

Unopened ampoules should be stored at -20ºC.
For economy of use, it is recommended that the solution be sub divided into aliquots and stored at -40ºC or below. Avoid repeated thawing/freezing.
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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Dissolve the total contents of the ampoule in 1.0ml of sterile distilled water. This solution will contain TNF receptor II Fc fusion protein (etanercept) at a concentration of 10,000 International Units/ml. Use carrier protein where extensive dilution is required.

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. It is the policy of WHO not to assign an expiry date to their international reference materials. 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. 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

This standard was produced under WHO guidelines cited in the WHO Technical Reports Series, No. 932, 2006, Annex 2.

Report on a Collaborative Study for Proposed 1st International Standard for TNF receptor II Fc fusion protein (Etanercept) WHO/BS/2015.2257

10. ACKNOWLEDGEMENTS

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UK Official Medicines Control Laboratory

World Health Organization

collaborative study for their contribution in evaluating the candidate preparations.

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

Physical and Chemical properties	
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not Physical appearance: Freeze dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	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: 4.6g
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.