Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Recombinant soluble transferrin receptor (rsTfR)
NIBSC code: 07/202
Instructions for use
(Version 1.0, Dated 23/03/2010)

1. INTENDED USE

Preparation 07/202 is intended to be used to standardise immunoassays for the measurement of serum transferrin receptor (sTfR).

Cellular uptake of iron bound to its carrier protein transferrin (Tf) is mediated by the transferrin receptor (TfR) [1-3]. A truncated, soluble form of the receptor is present in serum (sTfR) [4], formed as a result of protease action. The sTfR circulates as a complex with transferrin which is present at approximately 250 times the concentration of the sTfR in molar terms. TfR density is upregulated when there is increased erythropoeisis and in iron deficiency. As the sTfR concentration correlates with the total TfR content, a raised sTfR concentration is therefore a marker of iron deficiency. The joint WHO/CDC Technical Consultation on Assessment of Iron Status at Population level (Geneva, April 6-8, 2004) concluded that measurement of both serum ferritin and the serum transferrin receptor (sTfR) provides the best approach for estimating the iron status of populations [5].

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

21.7 mg/L or 303 nmol/L (when reconstituted with 0.50 mL distilled or deionised water). These values apply to free rsTfR monomer.

4. CONTENTS

Country of origin of biological material: United Kingdom.
rsTfR was prepared from the portion of the human TfR gene encoding residues 121-760 (the C-terminal amino acid of wild-type TfR) by Caltech (CA, USA) [6]. The choice of the N-terminal start site for rsTfR had been based on studies of a previously characterised soluble fragment produced by trypsin digestion of placental TfR [7]. In common with sTfR, the trypsin fragment and rsTfR have been reported to form a stable dimer binding 2 molecules of transferrin [6, 8]. Mass spectrometry of rsTfR revealed a major peak of 78,336 Da. Analysis of peptides cleaved from an SDS-PAGE-derived rsTfR spot using MALDI-TOF confirmed mass identity with equivalent theoretical peptides from published TfR sequence data [9, 10]. The rsTfR was shown to be glycosylated by a mobility shift of approximately 7,000 Da upon SDS-PAGE of rsTfR treated with PNGase F to remove N-linked glycans compared to untreated rsTfR.

The concentration of rsTfR was determined from the A280nm and using the adjusted theoretical extinction coefficient and molecular weight calculated from its published sequence [6, 9, 10; molecular weight = 71,725; extinction coefficient = 93,790; 1 mg/mL solution therefore has an absorbance of 1.308]. The rsTfR was diluted to 21.74 mg/L in transferrin receptor-depleted human serum that had been tested and found negative for anti-HIV I and II, HBsAg and anti-HCV (SCIPAC, Sittingbourne, Kent, UK; sTfR was not detected in the depleted serum in immunoassays kindly performed by Dade Behring), dispensed in glass ampoules at 4°C (~0.5 mL/ampoule), and lyophilised. The mean weight of the dispensed solution in 82 ampoules was 0.5062 g. The imprecision of the filling (CV) was 0.24%, the oxygen head space was 0.97%, and the residual moisture was 0.54%.

5. STORAGE

Store unopened ampoules at -20°C or below.
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.

Reconstitute the ampoules contents with 0.50 mL distilled or deionised water. The reconstituted material should be used to standardise assays for the serum transferrin receptor (sTfR).

Preparation 07/202 was subjected to an international collaborative study involving 6 commercial immunoassay kits which showed that measurement of the sTfR content of three serum samples relative to 07/202, rather than against kit calibrators, markedly improved agreement between different assay methods.

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.

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

Accelerated degradation studies on 07/202 are ongoing, but the estimated % loss per year for an earlier trial fill of rsTfR in sTfR-depleted serum is 0.05%, which represents very good stability.

9. REFERENCES

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World Health Organization

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10. ACKNOWLEDGEMENTS

We thank the participants of the collaborative study.

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: lyophilisate		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	Unknown
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains human serum		

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 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: 0.04g
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.