



Non WHO Reference Material
Anti-Ferritin Monoclonal Antibodies 1 and 2
NIBSC code: 87/654 & 87/662
Instructions for use
(Version 4.0, Dated 08/04/2008)

This material is not for in vitro diagnostic use.

1. INTENDED USE

These materials are intended as reference reagents for the immunoassay of serum ferritin.

2. CAUTION

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

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.

87/662:

This preparation contains material of human origin. The freeze-dried preparation has been tested and found negative for HB_sAg, anti-HIV and HCV RNA by PCR.

87/654:

This preparation does not contain material of human origin.

3. UNITAGE

N/A

4. CONTENTS

Country of origin of biological material: UK.

Anti-ferritin monoclonal antibody 1, 87/654 (coating antibody)

Anti-ferritin monoclonal antibody 1 was freeze-dried in the presence of 0.2% (w/v) trehalose. Open the ampoule according to the instructions below and reconstitute the contents with 1.0 ml of distilled water. Dilute 1/250 in 0.05 M carbonate buffer, pH 9.6, for use, e.g dilute 100µl to 25 ml - this is sufficient for 1 plate.

Anti-ferritin monoclonal antibody 2, 87/662 (HRP-labelled antibody)

Anti-ferritin monoclonal antibody 2 was freeze-dried in the presence of 5% (w/v) HSA. Open the ampoule according to the instructions below and reconstitute the contents with 1.0 ml of distilled water. Dilute 1/200 in PBS containing 0.05% (v/v) Tween and 0.5% (w/v) BSA for use, e.g dilute 100µl to 20 ml - this is sufficient for 1 plate.

5. STORAGE

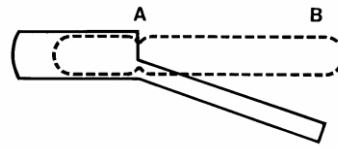
Store unopened ampoules at -20°C or below. The reconstituted antibodies should be stored at 4°C for up to 1 week. Do not store diluted antibodies.

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

TWO SITE ELISA FOR SERUM FERRITIN

SUMMARY

1. Add 200 µl standard or serum diluted in PBST/BSA to each well of an anti-ferritin-coated microtitre plate.
2. Incubate at room temperature for 120 minutes. Empty plate and wash three times with PBST.
3. Add 200 µl diluted HRP conjugate to each well.
4. Repeat step 2.
5. Add 200 µl OPD substrate and incubate for 30 minutes in the dark.
6. Add 50 µl 25% H₂SO₄.
7. Read absorbance within 30 minutes at 492 nm.

Materials

All of the following reagents must be of analytical grade.

Sodium carbonate, Na₂CO₃

Sodium bicarbonate, NaHCO₃

Bovine serum albumin (BSA; globulin-free, Sigma #A7030)

Sodium Chloride, NaCl

Potassium Chloride, KCl

Disodium phosphate, Na₂HPO₄ (anhydrous)

Potassium phosphate, KH₂PO₄ (anhydrous)

Tween 20 (polyoxyethylene sorbitan monolaurate, Sigma #P-1379)

Citric acid monohydrate, C₆H₈O₇·H₂O

Hydrogen peroxide, H₂O₂ (30%)

O-phenylenediamine dihydrochloride (OPD) (Sigma #P1526)

Sulphuric acid, H₂SO₄

NOTE: If Sigma reagents are not available use equivalent grade from elsewhere. Other hydrated phosphates may also be used but recalculate the amounts required.

Reagents

Phosphate buffered saline (PBS), 0.15 M, pH 7.2: Prepare a 10 x concentrated (1.5 M) stock solution by dissolving the following in distilled water: sodium chloride, 80 g; potassium chloride, 2 g; sodium phosphate (di-basic), 11.5 g; and potassium phosphate (mono-basic) 2 g. Dilute to 1 litre with distilled water. Store at room temperature. A pH of 7.2 is not achieved until this stock solution is diluted to 1 x concentration.

Prepare a **working PBS-Tween (PBST) buffer** (0.15 M) by diluting 100ml concentrated PBS to 1 litre with distilled water and adding 0.5ml Tween 20. May be stored at 4°C for up to 2 weeks.

Phosphate buffered saline containing 0.5% BSA (PBST/BSA): Prepare by adding 0.5 g BSA to 100ml working PBST. May be stored at 4°C for up to 2 weeks.

Carbonate buffer, 0.05M; pH 9.6: Dissolve sodium carbonate, 1.59 g and sodium bicarbonate, 2.93 g in 1 litre distilled water. Store at room temperature.



Coating of plates

Coat plates with diluted 87/654 (250 µl/well) diluted in 0.05 M carbonate buffer, pH 9.6. Incubate the plates overnight at 4°C. On the day of the assay, remove the coating reagent and block unreacted sites by adding 250 µl 0.5% (w/v) BSA (Sigma A-7030) in 0.05 M carbonate buffer, pH 9.6. After 30 minutes incubation at room temperature, wash each plate three times with 300 µl PBST, allowing each wash to stand for 3 minutes at room temperature.

Preparation of Working Standards

Dilute the reconstituted standard (e.g. 3rd IS for ferritin, recombinant, 94/572) to a ferritin concentration of 100 µg/l in 0.15 M phosphate buffered saline, pH 7.2, containing 0.05% (v/v) Tween 20 and 0.5% (w/v) BSA (PBST/BSA). Prepare working standards by diluting this stock solution to concentrations of 2, 5 and 10 µg/l. Dilute each of these 1:10 in PBST/BSA to obtain a series of working standards containing 0.2, 0.5, 1.0, 2.5 and 10 µg/l. In addition, use a blank (0 µg/l) containing PBST/BSA alone.

Sera

Assay in duplicate by adding 200 µl of serum diluted 20 x in PBST/BSA.

Conjugate

Dilute in 1% BSA in PBST-T for the assay.

ELISA Protocol

The method is outlined above. We recommend the use of a multi-channel pipette for rapid addition of buffers to the wells. It is important that all standards and sera are added to each plate within 30 minutes.

Cover the plate with an adhesive-backed acetate plate sealer or parafilm and incubate for 2 hours at room temperature. Empty the wells by sharply inverting the plate and dry by tapping briefly on paper towels. Wash three times with 300µl PBST, allowing each wash to stand for 3 minutes at room temperature.

After the addition of 200 µl diluted HRP conjugate (87/662) to each well, incubate the plate for a further 2 hours at room temperature. Repeat the washing step. Add 200 µl substrate solution to each well. This contains 34 mg O-phenylene diamine (OPD: Sigma P-1526) in 100 ml citrate-phosphate buffer, 0.15 M, pH 5.0 with 0.01% (v/v) hydrogen peroxide. Incubate the plate for 30 minutes at room temperature in the dark. Terminate the colour reaction by adding 50 µl 25% (v/v) sulphuric acid to each well. Read the absorbance at 492 nm within 30 minutes using an automatic plate reader. Use PBS as instrument blank.

8. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. It is the policy of WHO not to assign expiry dates to their international reference materials. They remain valid until withdrawn or amended. Accelerated degradation studies have indicated that these preparations are suitably stable, when stored at -20°C or below.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. 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

M Worwood, SJ Thorpe, A Heath, CH Flowers, JD Cook. Stable lyophilised reagents for the serum ferritin assay. Clin Lab Haematol 13, 297-305 (1991).

10. ACKNOWLEDGEMENTS

N/A

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: 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 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: 0.08g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. Attached: No