



**WHO Reference Reagent
Haemoglobin F Lysate, Raised
NIBSC code: 85/616
Instructions for use
(Version 4.0, Dated 04/04/2008)**

1. INTENDED USE

This material (in ampoules coded 85/616) is intended for the control of test procedures used in the diagnosis of beta thalassaemia trait and delta beta thalassaemia trait.

The material has been subjected to an international collaborative study following which it was established by the World Health Organization as the International Reference Reagent for Haemoglobin F Lysate, Raised, with an assigned value of 3.4% HbF of the total haemoglobin present [1].

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.

Do not breathe fumes as the material contains cyanide.

3. UNITAGE

HbF content of 3.4% of the total haemoglobin present.

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content and was determined to be +/- 0.31%.

4. CONTENTS

Country of origin of biological material: United Kingdom.

The material has been produced by freeze-drying a solution of haemoglobin prepared from human red cells and which has been converted to cyanmethaemoglobin and stabilised by the addition of potassium ferricyanide (1.3 mM), potassium cyanide (6.5 mM) and chloramphenicol (1 mg/ml). The Haemoglobin F is in a matrix of Haemoglobin A and Haemoglobin F which are present in similar proportions to those found in people with beta thalassaemia trait.

DO NOT BREATHE FUMES AS THE MATERIAL CONTAINS CYANIDE.

5. STORAGE

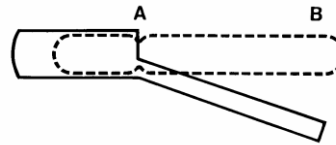
Unopened ampoules should be stored at -20°C.

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

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

The material should be reconstituted by the addition of 5 ml distilled water which produces a clear red solution. After reconstitution the material should be used within 24 hours. It should not be refrozen. The total haemoglobin concentration of this solution was found to be approximately 5 g/l which is satisfactory for the first step in the two minute alkali denaturation method for quantitating Hb F [2].

After reconstitution this solution should be used in the same way as the cyanmethaemoglobin solution prepared in the first step of the Betke two minute alkali denaturation technique for quantitating Haemoglobin F [3, 4] or a modification of that method [5].

8. STABILITY

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. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

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

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

1. WHO Technical Report Series 1994.
2. ICSH Recommendations for fetal haemoglobin reference preparations and fetal haemoglobin determination by the alkali denaturation method. British Journal of Haematology, 1979, 42, 133-136.
3. Betke K, Marti H R and Schlicht I. Estimation of small percentages of fetal haemoglobin. Nature, 1959, 184, 1877-1878.
4. Lehmann H and Huntsman R G. Man's Haemoglobins. 2nd Ed 1974, North Holland Publishing Co, Oxford, pp 405-406.
5. Pembrey M E, McWade P and Weatherall D J. Reliable routine estimation of small amounts of fetal haemoglobin by alkali denaturation. Journal of Clinical Pathology, 1972, 25, 738-740.

10. ACKNOWLEDGEMENTS

These materials were provided by Dr AD Stephens and Dr BJ Wild of the Department of Haematology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, who also organised 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: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin. Contains cyanide. Do not breathe fumes.
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 : 0.1g
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_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.