



**WHO International Standard
Minimum Potency Of Anti-D Blood Grouping Reagents
NIBSC code: 99/836
Instructions for use
(Version 3.0, Dated 04/04/2008)**

1. INTENDED USE

The quality of blood grouping reagents is clearly an important factor for safe blood transfusion. Preparation 99/836 is intended to define, by international consensus, a recommended minimum potency of anti-D blood grouping reagents in tube tests [WHO/BS/04.2000].

WHO/BS/04.2000: Summary of contents of document

The international standard for minimum potency of anti-D blood grouping reagents, code 99/836, was evaluated against a wide range of commercial anti-D blood grouping reagents in an international collaborative study involving 20 laboratories in 13 countries. Laboratories titrated 99/836 in parallel with as many commercial anti-D blood grouping reagents as were available to them according to specified haemagglutination methodology. The ratios of the mean endpoint titres of the reagents to that of 99/836 within each laboratory were calculated. By international consensus, a 1 in 3 dilution of the reconstituted contents of 99/836 was deemed appropriate to define the minimum acceptable potency of low protein (i.e. monoclonal IgM) anti-D blood grouping reagents. A 1 in 8 dilution of the reconstituted contents of 99/836 was deemed appropriate to define the minimum acceptable potency of high protein (e.g. polyclonal) anti-D blood grouping reagents.

This preparation is not for use as a blood grouping reagent, nor as a reference preparation for the specificity of anti-D blood grouping reagents.

2. CAUTION

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

This preparation is lyophilized culture supernatant containing a human IgM monoclonal anti-D. 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

N/A

4. CONTENTS

Country of origin of biological material: United Kingdom.

This preparation is lyophilized culture supernatant containing a human IgM monoclonal anti-D (RUM-1; kindly donated by Serologicals Inc., Livingston, UK). The imprecision of the filling (coefficient of variation) was 0.12%, and the residual moisture content was 1.4%.

5. STORAGE

Store unopened ampoules 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

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 manufacturer's 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 contents with 1.0 ml distilled/deionized water.

A 1 in 3 dilution of the reconstituted contents of 99/836 defines the recommended minimum acceptable potency of low protein (i.e. monoclonal IgM) anti-D blood grouping reagents.

A 1 in 8 dilution of the reconstituted contents of 99/836 defines the recommended minimum acceptable potency of high protein (e.g. polyclonal) anti-D blood grouping reagents.

For parallel titrations of 99/836 and low protein anti-D blood grouping reagents

Make a 1 in 3 dilution of reconstituted 99/836 in buffered saline containing 2% (w/v) BSA i.e. mix 1 volume reconstituted contents with 2 volumes of buffered saline containing 2% (w/v) BSA. This is the starting concentration of 99/836 for comparison with low protein anti-D blood grouping reagents.

Prepare doubling dilution series of 99/836 (from the 1 in 3 pre-dilution) and the blood grouping reagent (from neat), using buffered saline containing 2% (w/v) BSA as diluent. Mix one volume of each starting concentration and dilution with one volume of a 2% (v/v) suspension of R₁r cells (pooled from 4 donors) in glass test tubes, and incubate the contents at RT (19-25°C) for 5 min.

Centrifuge the tests for 1 min at approximately 1000 rpm (100-125 g) or for 15 sec at approximately 3400 rpm (900-1000 g) or for a time and at a speed appropriate for the centrifuge being used, or for the shortest time at the lowest speed recommended by the reagent manufacturer. Re-suspend the cell button by gentle agitation and macroscopically grade the reaction according to conventional criteria.

For parallel titrations of 99/836 and high protein anti-D blood grouping reagents

Make a 1 in 8 dilution of reconstituted 99/836 in buffered saline containing 2% (w/v) BSA i.e. mix 1 volume reconstituted contents with 7 volumes of buffered saline containing 2% (w/v) BSA. This is the starting concentration of 99/836 for comparison with high protein anti-D blood grouping reagents. Prepare doubling dilution series of 99/836 (from the 1 in 8 pre-dilution) using buffered saline containing 2% (w/v) BSA as diluent. Prepare doubling dilution series of high protein reagents using the manufacturer's specified Rh control or buffered saline containing 22% (w/v) BSA as diluent. Mix one volume of each starting concentration and dilution with one volume of a 2% (v/v) suspension of R₁r cells (pooled from 4 donors) in glass test tubes, and incubate the contents at RT (19-25°C) for 5 min.

Centrifuge the tests for 1 min at approximately 1000 rpm (100-125 g) or for 15 sec at approximately 3400 rpm (900-1000 g) or for a time and at a speed appropriate for the centrifuge being used, or for the shortest time at the lowest speed recommended by the reagent manufacturer.

Re-suspend the cell button by gentle agitation and macroscopically grade the reaction according to conventional criteria.

ANTI-D BLOOD GROUPING REAGENTS FOR USE IN TUBE TESTS SHOULD HAVE A POTENCY TITRE AT LEAST EQUAL TO THAT OF 99/836, WHEN RECONSTITUTED AND PRE-DILUTED AS SPECIFIED ABOVE.

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 or recommended usage 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. Once reconstituted, users should determine the stability of the material according to their own conditions of storage and use.

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

Accelerated degradation studies on 99/836 indicate that the lyophilized material will be adequately stable at -20°C.

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

9. REFERENCES

SJ Thorpe, B Fox, AB Heath, M Scott, M de Haas, S Kochman and A Padilla. An International Standard for minimum potency of anti-D blood grouping reagents: evaluation of a candidate preparation in an international collaborative study. Vox Sang 90, 131-139 (2006).

10. ACKNOWLEDGEMENTS

We thank Serologicals Inc. for donating the culture supernatant, and 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: 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: 0.01g
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.