WHO International Standard
Minimum Potency of Anti-A and Anti-B Blood Grouping Reagents
NIBSC code: 03/188 & 03/164
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. Preparations 03/188 and 03/164 are intended to
define, by international consensus, the recommended minimum potency
of anti-A and anti-B blood grouping reagents, respectively, in tube tests.

The international minimum potency standards for anti-A and anti-B blood
grouping reagents, codes 03/188 and 03/164, respectively, were evaluated against a wide range of commercial anti-A and anti-B blood
grouping reagents, respectively, in an international collaborative study
involving 16 laboratories in 9 countries. Laboratories titrated 03/188 and
03/164 in parallel with as many commercial anti-A and anti-B reagents as
were available to them according to specified haemagglutination methodology. Comparisons were also made with existing reference
preparations for anti-A and anti-B (WHO IS, CBER/FDA reference
preparations and British minimum potency reference preparations). The
eratios of the mean endpoint titres of the anti-A or anti-B reagents or
reference preparations to those of 03/188 and 03/164, respectively, within
each laboratory were calculated. By international consensus, a 1 in 8
dilution of the reconstituted contents of 03/188 was deemed appropriate to
define the minimum acceptable potency of anti-A blood grouping
reagents. A 1 in 4 dilution of the reconstituted contents of 03/164 was
deemed appropriate to define the minimum acceptable potency of anti-B
blood grouping reagents.

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

Preparations 03/188 and 03/164 each contain the lyophilized residue of
culture supernatant containing murine monoclonal antibodies. 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.

Preparations 03/188 and 03/164 each contain the lyophilized residue of 1
ml culture supernatant containing monoclonal IgM anti-A (BRIC 131;
kindly donated by the IBGRL, Bristol, UK) and anti-B (ES4; kindly donated
by Alba Bioscience, Edinburgh, UK), respectively. For the anti-A, the
imprecision of the filling (coefficient of variation) was 0.08%, and the
residual moisture content was 0.8%; for the anti-B, the imprecision of the
filling (coefficient of variation) was 0.08%, and the residual moisture
content was 1.2%.

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.

Tap the ampoule gently to collect the material at the bottom (labeled)
end. Ensure that the disposable ampoule safety breaker provided is
pushed down on the stem of the ampoule and against the shoulder of the
ampoule body. Hold the body of the ampoule in one hand and the
disposable ampoule breaker covering the ampoule stem between the
thumb and first finger of the other hand. Apply a bending force to open
the ampoule at the coloured stress point, primarily using the hand holding
the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that
might enter the eyes, for example, by the use of suitable gloves and an
eye shield. Take care that no material is lost from the ampoule and no
glass falls into the ampoule. Within the ampoule is dry nitrogen gas at
slightly less than atmospheric pressure. A new disposable ampoule
breaker is provided with each DIN ampoule.

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

RECONSTITUTE THE AMPOULE CONTENTS WITH 1.0 ML DISTILLED
OR DEIONISED WATER.

Allow several minutes at RT, with occasional vortexing, for reconstitution.

A 1 in 8 dilution of the reconstituted contents of 03/188 defines the
recommended minimum acceptable potency of anti-A blood grouping
reagents, in tube tests.

A 1 in 4 dilution of the reconstituted contents of 03/164 defines the
recommended minimum acceptable potency of anti-B blood grouping
reagents, in tube tests.

For parallel titrations of 03/188 or 03/164 and anti-A or anti-B blood
grouping reagents, respectively:

Make a 1 in 8 dilution of reconstituted 03/188 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 03/188 for comparison with anti-A blood grouping reagents.

Make a 1 in 4 dilution of reconstituted 03/164 in buffered saline containing
2% (w/v) BSA i.e. mix 1 volume reconstituted contents with 3 volumes of
buffered saline containing 2% (w/v) BSA. This is the starting concentration
of 03/164 for comparison with anti-B blood grouping reagents.

Prepare doubling dilution series of 03/188 (from the 1 in 8 pre-dilution) and
the anti-A 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 A or
A,B cells in glass test tubes, and incubate the contents at RT (19-25°C)
for 5 min.

Prepare doubling dilution series of 03/164 (from the 1 in 4 pre-dilution) and
the anti-B 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 B or
A,B cells 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. Ensure that the
centrifugation does not result in an excessive force being necessary to
dislodge the cell button prior to observation. Re-suspend the cell button by
gentle agitation and macroscopically grade the reaction according to
conventional criteria.

ANTI-A BLOOD GROUPING REAGENTS FOR USE IN TUBE TESTS
SHOULD HAVE A POTENCY TITRE AT LEAST EQUAL TO THAT OF
03/188 WHEN RECONSTITUTED AND PRE-DILUTED AS SPECIFIED ABOVE.

ANTI-B BLOOD GROUPING REAGENTS FOR USE IN TUBE TESTS SHOULD HAVE A POTENCY TITRE AT LEAST EQUAL TO THAT OF 03/164 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 03/188 and 03/164 indicate that the lyophilized materials 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

10. ACKNOWLEDGEMENTS
We thank The International Blood Group Reference Laboratory and Alba Bioscience for the culture supernatants, and the participants of the collaborative study.

11. FURTHER INFORMATION
Further information can be obtained as follows:
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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
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<tbody>
<tr>
<td><strong>Physical appearance:</strong></td>
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<tr>
<td><strong>Stable:</strong></td>
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<tr>
<td><strong>Hygroscopic:</strong></td>
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<tr>
<td><strong>Flammable:</strong></td>
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<tr>
<td><strong>Other (specify):</strong></td>
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</tbody>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
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<tr>
<td><strong>Effects of inhalation:</strong></td>
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<tr>
<td><strong>Effects of ingestion:</strong></td>
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<tr>
<td><strong>Effects of skin absorption:</strong></td>
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</tbody>
</table>

**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
- **Toxicity Statement**: Toxicity not assessed
- **Veterinary certificate or other statement if applicable**: No
- **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_eftstandardsrev2004.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.