Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**WHO International Standard**
**1st International Standard for the biological activities of Rituximab**
**NIBSC code: 14/210**
**Instructions for use**
**(Version 1.0, Dated 06/11/2017)**

## 1. INTENDED USE

The World Health Organisation (WHO) Expert Committee on Biological Standardisation (ECBS) recognised the need for a reference standard to evaluate the performance of potency assays used to assess the biological activities of rituximab. The preparation 14/210 has been evaluated in an international multi-centre collaborative study (described in section 3). Based on the data of this study the preparation has been formally adopted by ECBS as the 1st WHO international standard for the in vitro biological activities of rituximab. The standard is intended to support the characterisation, calibration and validation of bioassays used to assess the biological activitites of rituximab and support the establishment of in house bioassay standards.

It should be noted that the bioactivity unitage of the standard is not intended to define specific activity for regulatory purposes neither to describe labelling or dosage of rituximab products. Further, the properties and characteristics of the material used to make this reference standard, such as purity and specific biological activity (U/mg) are not intended to serve any regulatory role in defining biosimilarity, and should not be extrapolated, calculated or inferred as serving this purpose in anyway whatsoever.

## 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

The preparation has been assigned the following arbitrary unitage:
1,000 international units (IU) of complement dependent cytotoxic (CDC) activity per ampoule
1,000 IU of antibody-dependent cell-mediated cytotoxic (ADCC) activity per ampoule
1,000 IU of cell-binding activity per ampoule
1,000 IU of apoptotic activity per ampoule

The bioactivity of the preparation was established using CDC, ADCC, cell-binding and apoptosis assays, respectively, using CD20-expressing target cell lines (WIL2-S, Raji, Z-138, Daudi and Jeko). The study included data from 16 laboratories for CDC, 11 laboratories for ADCC, 5 laboratories for cell binding assays and one laboratory for apoptosis assay from 9 different countries. Participants evaluated the preparation using their qualified in house bioassay platforms. Data was returned for viability, cytotoxicity, apoptosis and/or reporter gene and binding assay results.

## 4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule is the lyophilised residue of a 1 mL solution containing approximately 100 µg rituximab protein, 25 mM tri-sodium citrate dehydrate, 150 mM sodium chloride, 1% human serum albumin, pH 6.5.

Note that the quantity in mass of rituximab is given as approximate for guidance, however, it is not a formally assigned content and should not be used to calculate or infer a specific biological activity.

## 5. STORAGE

Unopened ampoules should be stored at -20 ºC.
**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
Dissolve the total contents of the ampoule in 1.0 mL of sterile destilled water. This solution will contain rituximab protein at a concentration of 1,000 IU/mL of CDC activity, 1,000 IU/mL ADCC activity, 1,000 IU/mL of cell-binding activity and 1,000 IU/mL of apoptotic activity.

## 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. Thus, no expiring date is assigned to international reference materials. Accelerated degradation studies have indicated that this material is suitably stable when stored at the recommended -20 ºC or below, for the assigned values. Once reconstituted, diluted or alliquoted, 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

This standard was produced under WHO guidelines cited in the WHO Technical Reports Series, No. 932, 2006, Annex 2.

Report on a collaborative study for proposed 1st WHO international standard for the biological activities of rituximab is available in: http://www.who.int/biologicals/expert_committee/BS2309_1st_International_Standard_for_Rituximab.pdf?ua=1

Prior, S., Hufton, S.E., Fox, B., Dougall, T., Rigsby, P., Bristow, A. and participants of the study. International standards for monoclonal antibodies to support pre- and post- marketing product consistency: Evaluation of a candidate international standard for the bioactivities of rituximab. MABS, 2017. http://www.tandfonline.com/doi/full/10.1080/19420862.2017.1386824

## 10. ACKNOWLEDGEMENTS

We are grateful to Sandoz GmbH for their generous donation of the rituximab material to develop this preparation. We are thankful to all the participants of the collaborative study for their contribution in evaluating the candidate preparation.

## 11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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 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 ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water.<br>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

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|---|
| **Country of origin for customs purposes*:** United Kingdom<br>* 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:** 1.0 G |
| **Toxicity Statement:** Toxicity not assessed |
| **Veterinary certificate or other statement** if applicable**.**<br>**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.