Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**WHO International Standard**
**Monoclonal Antibody for Serotyping Bordetella pertussis Fimbrial Antigen 2 (1st WHO IS)**
**NIBSC code: 06/124**
**Instructions for use**
**(Version 4.0, Dated 04/08/2016)**

## 1. INTENDED USE

This reagent coded 06/124 is a monoclonal antibody of murine origin which reacts to B. pertussis fimbriae serotype 2. In 2009 on the basis of the results of a collaborative study (WHO/BS/09.2120) preparation 06/124 has been established (3) as the First International Standard for Serotyping Bordetella pertussis Fimbrial Antigen 2.

## 2. CAUTION

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

The material is of bovine origin. The material is certified to be obtained from animals taken from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having BSE & which has not been fed rations containing ruminant derived protein during that period. 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

No unitage is assigned to this material.

## 4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule of 06/124 contains the freeze dried powder from 1ml of concentrated cell culture supernatant, adjusted with phosphate buffered saline to give a concentration of 10mg/ml IgG
as determined by UV at 480nm.
The bovine source is from the use of foetal bovine serum in the culturing media.

## 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

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
The entire ampoule contents should be reconstituted with 1ml of saline, dispensed into suitable aliquots and stored at -20°C until needed. Repeat freeze thawing should be avoided. Data from an in- use stability study suggests that the reconstituted material can be used if it have been suitably stored, but this should be validated under individual laboratory conditions.
Suggested working dilutions, these may vary under different laboratory conditions:-

For slide agglutination: Dilutions in the range 1/10 to 1/50
For microplate assay: Dilutions in the range 1/100 to 1/800
For whole cell ELISA: Dilutions in the range 1/100 to 1/1000

Preparation of ampoules
The hybridoma cell line BPF2, for the production of this monoclonal antibody, was originally developed by Drs Brennan, Manclarke, and Li, CBER/FDA USA (1) and was used to produce a large volume of cell culture supernatant which was subsequently concentrated and freeze dried.

Collaborative Study
In a collaborative study involving eleven laboratories in nine countries, preparations 06/124 and 06/128 have been assessed for their suitability to serve as WHO International Standard Monoclonal Antibodies for B. pertussis serotyping, in comparison to current WHO Interim Reference Reagents for B.pertussis fimbriae 2 (coded 04/154) and 3 (coded 04/156), using a standard strain panel and 23 in-house strains. Microplate agglutination method, slide agglutination assay and ELISA based assays were performed by participants. Data for these assays showed good agreement between laboratories using all methods included in the study. Preparations 06/124 and 06/128 showed good specificity in all current typing methods, evidenced by >95% sensitivity on the corresponding strains and no cross-reaction. Comparison of the candidate materials with the WHO Interim Reference Reagents also showed good agreement among laboratories and on the basis of the results of this collaborative study 06/124 has been established (3) as the 1st International Standard for Serotyping Bordetella pertussis Fimbrial Antigen 2.

## 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.

This freeze dried material was found to be stable, with no detectable loss of activity by ELISA and functional assay after 2 years storage at 37°C.

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

Users who have any data supporting any change in the characteristics of this material are encouraged to contact NIBSC.

## 9. REFERENCES

1) Li et al. Inf. Immun. 56 (12) : 3184 – 3188

2) D. Xing, P. Newland and M. Corbel, Evaluation of purified monoclonal antibodies to Bordetella pertussis fimbriae type 2 and 3, WHO/BS/04.1998

3) D. Xing, P. Newland and M. Corbel
International Collaborative Study: Evaluation of Proposed International Standard Monoclonal Antibodies for serotyping Bordetella pertussis Serotype 2 and Serotype 3. WHO/BS/09.2120.

## 10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr B. Meade, FDA, USA for the kind transfer of the hybridoma cell line BPF2 for the production of this monoclonal antibody and to the Large Scale Laboratory (NIMR, MRC, UK) for preparation of the raw material.
We would like to express our thanks to Dr Paul Matejtschuk (NIBSC) for assistance in the determination of freeze drying conditions and for moisture and oxygen determinations of the ampouled material and the staff of CBRM for assistance with the filling procedure. We also thank all the participants for their helpful contribution to the study.

## 11. FURTHER INFORMATION

Further information can be obtained as follows;

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

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: Coloured freeze-dried powder | | Corrosive: | No |
| Stable: | Yes | Oxidising: | No |
| Hygroscopic: | No | Irritant: | No |
| Flammable: | No | Handling:See caution, Section 2 | |
| Other (specify): | Contains material of biological 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

| |
|---|
| **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:** Non-toxic |
| **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.