



Non WHO Reference Material
NIBSC Research Reagent Cocksfoot (*Dactylis glomerata*)
Pollen Extract
NIBSC code: 75/506
Instructions for use
(Version 6.0, Dated 24/07/2013)

This material is not for in vitro diagnostic use.

1. INTENDED USE

This standard is comprised of a batch of ampoules coded 75/506. Each ampoule contains the residue after freeze drying of approximately 1.0ml of cocksfoot pollen extract.

2. CAUTION

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

The material is not of human or bovine origin. 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

A potency of 100 units has been assigned to each ampoule.

4. CONTENTS

Country of origin of biological material: United Kingdom.

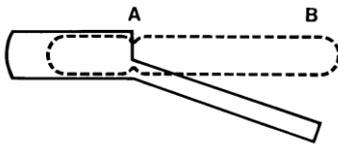
5. STORAGE

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 total contents of the ampoule should be reconstituted with 1.0ml distilled water and dissolved gently by swirling to avoid froth. The reconstituted reagent should be used as soon as possible after reconstitution

Biological Activity.

In vitro comparison

On iso-electric focussing, (pH 3.0-11.0 at 220V in 7% polyacrylamide gel) 17 bands staining with protein dyes developed, and all bands were identical in the parent material and the freeze dried standard.

The standard can also be used in in vitro assays such as the radioallergosorbent test (RAST), RAST inhibition, quantitative immunoelectrophoresis (CIE & CRIE), SDS-PAGE and immunoblotting.

In vivo comparison

Inbred Hooded rats with no previous exposure to cocksfoot pollen were used to assay non-immunological vaso-active mediator release. Falling concentrations from 350 micrograms to 0.05 micrograms contained in 0.05ml physiological saline were injected intradermally into the shaved skin of the animals. After 3 hours, Evans-blue dye was injected intravenously and the sites watched for a change of vascular permeability. Marked reactions occurred at 350 micrograms and diminished thereafter. The end points of the titration were 50 micrograms for the original 25 micrograms for the freeze dried preparation.

The test was repeated in a rhesus monkey, where identical end-points were obtained.

When these doses were used to challenge animals sensitised intradermally with homologous anti-cocksfoot serum by passive transfer, strong positive results were obtained.

It is therefore recommended that for intradermal testing in laboratory animals, an appropriate challenge dose of the lyophilised material containing about 25 micrograms (4 units) in 0.05ml physiological saline is used.

Bulk Material

The standard was produced by extraction of approximately 600gm of cocksfoot pollen (Batch BX 21922, harvested in 1974) in 6 litres of phenol saline at neutral pH for 24 hours.

The extract was filtered to give a final volume of 5,500ml, and this solution was then dialysed against distilled water using two 'Minicoil' haemodialysis coils and standard flow rates (150ml/min for extract, 400ml/min for water). After dialysis, the solution was again filtered through disc of pore size 0.12 micrometres. A 100 ml sample was removed and tested for sterility and quality. The bulk of the solution of extract was stored at 5°C until distributed into ampoules and lyophilised.

Samples of the extract (parent material) were retained at 5°C for comparison with the freeze dried standard.

Distribution into ampoules

At NIBSC the extract was distributed in 1.0ml volumes at room temperature into approximately 4000 ampoules, coded 75/506. The mean weight of liquid contents of 54 checkweight ampoules taken at intervals during the fill was 1.010gm +/- 0.25%. The contents of the ampoules were then freeze dried and secondarily desiccated under the conditions normally used for international biological standards¹. The mean dry weight was 6.44 micrograms (n=6) and moisture content was 0.58% (n=6).

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.

Accelerated degradation studies have shown freeze dried allergen extracts stored in unopened ampoules at -20°C to be extremely stable over a number of years.

9. REFERENCES

1) Campbell, P.J., International Biological Standards and Reference Preparations. II. Procedures used for the production of biological standards and reference preparations. J. Biol. Stand. 2: 259-267 (197).

10. ACKNOWLEDGEMENTS



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: 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. 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

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

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: 6.44 µg

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable.

Attached: No