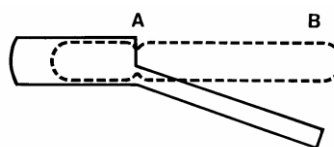




International Ref. Preparation
Papain (92/658) & anti-D (91/562)
NIBSC code: 92/658 & 91/562
Instructions for use
(Version 5.0, Dated 22/01/2013)



1. INTENDED USE

These materials are distributed on behalf of the International Society for Blood Transfusion/International Committee for Standards in Haematology (ISBT/ICSH).

The overall performance of any enzyme preparation is a product of its proteolytic potency and the method by which it is used. The efficacy of a particular enzyme preparation should be judged by the sensitivity of true positive reactions and freedom from false-positive reaction achieved in the technique for which it is recommended.

Papain batch 92/658 is equivalent to papain batch 94/676 previously distributed for this use.

The efficacy of a particular enzyme preparation and technique is compared to that of the ISBT/ICSH reference materials using a doubling dilution series of the reference anti-D 91/562 and six inert sera (2 group A, 2 group B and 2 group O), with a pool of at least four group O R₁r blood samples in a two-stage reference technique.

The anti-D 91/562 is not for use as a blood grouping reagent.

Further details can be found in the Reference (Section 9).

2. CAUTION

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

The preparation 91/562 contains material of human origin, which has been tested and found negative for HBsAg, HIV antibody, HCV antibody and HCV RNA by PCR. The preparation 92/658 does not contain material of human 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

N/A

4. CONTENTS

Country of origin of biological material: United Kingdom.

5. STORAGE

Store unopened ampoules at -20°C or below.

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

a) Reconstitution of freeze-dried materials

Reconstitute the whole contents of each ampoule as follows:-

Papain (92/658) – add 2ml of unbuffered 0.15M NaCl

Anti-D (91/562) – add 1ml of distilled/deionised water

Leave the ampoules at room temperature (19-25°C) for 15 minutes without shaking. Then, after gentle agitation, the reconstituted materials should be stored at 2-6°C and used within 24 hours.

b) Prepare a pool of washed packed red cells from equal proportions of at least four pooled group O R₁r blood samples.

c) Mix two volumes of reconstituted reference papain preparation (92/658) with one volume of packed red cells and incubate for 15 minutes at 37°C.

d) Wash the treated cells three times with phosphate buffered saline (10mM pH 7.2 phosphate buffer in 0.15M NaCl). Resuspend the treated cells to 3% (v/v) in PBS.

e) Prepare a doubling dilution series from neat to 1/512 of the reconstituted reference anti-D 91/562 in PBS containing 2% (w/v) bovine serum albumin.

f) Mix one volume of 3% treated cells with one volume reference anti-D and one volume PBS in a glass tube. Repeat for each dilution of Anti-D.

g) For each of six inert sera (2 group A, 2 group B, 2 group O), mix one vol 3% treated cells with one vol of inert serum and one vol of PBS.

h) Incubate for 15 minutes at 37°C.

i) Centrifuge tubes at 500rcf for 15 seconds.

j) Gently resuspend the cells and assess the degree of agglutination macroscopically.

k) In parallel, test the pooled cells with the titration series of the anti-D 91/562 using the test enzyme in the test method.

l) Score the reaction grades as follows:-

Grade	Score	Observation
C	12	Cell button in one clump
+++	10	Cell button dislodges into several large clumps
++	8	Cell button dislodges into many small clumps
+	5	Cell button dislodges into granular but definite clumps
(+)	3	Cell button dislodges into fine small granules
0	0	Cell button dislodges into separate clumps

m) Sum the reaction scores for each titration and sum the inert sera reaction scores for each enzyme.

EVALUATION



When the test enzyme preparation is used by its recommended technique:

- The sum of the scores of reactions with six inert sera should not exceed the sum obtained using the reference papain preparation (92/658) in the reference technique
- The titration score with the anti-D 91/562 should attain or exceed that obtained when using the reference papain preparation (92/658) in the reference technique.

8. STABILITY

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 its reference materials. They remain valid with the assigned potency 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. For information specific to a particular biological standard, contact NIBSC (see email address below), or where known, the appropriate NIBSC scientist.

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

9. REFERENCES

Review of the problems involved in using enzymes in blood group serology - provision of freeze-dried ICSH/ISBT protease enzyme and anti-D reference standards. Scott ML, Voak D, Phillips PK, Hoppe PA, Kochman SA. Vox Sanguinis 1994; 67: 89-98.

10. ACKNOWLEDGEMENTS

N/A

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.

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.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

No IEP/2000: 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.			

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: 0.08g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable.
Attached: No