



**Non WHO Reference Material
Corticotrophin (ACTH), Human
NIBSC code: 74/555
Instructions for use
(Version 5.0, Dated 23/09/2010)**

This material is not for in vitro diagnostic use.

This consists of a batch of ampoules (coded 74/555) containing human corticotrophin (ACTH) intended for reference purposes, although it has no status as an official standard.

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

6.2 IU/ampoule – by definition.

NOTE (added September 1983) * preliminary data had suggested that ampoules of 74/555 contained about 11.6µg of ACTH. However, more detailed study has shown that the actual ampoule content of ACTH is about 25µg. Since there is always uncertainty about the 'nominal content' of a reference preparation and in order to obviate further confusion, 74/555 has been assigned a unitage based on its potency by in vivo i.v. bioassay against the Third International Standard for Corticotrophin, Porcine for Bioassay (I.S.)¹.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the freeze-dried residue of 0.5ml of a solution which contained:

ACTH	approximately 25µg*
Acetic acid	approximately 3mg
Mannitol	approximately 2.5mg
Human plasma albumin	approximately 0.5mg
Sodium chloride	approximately 45µg

Nitrogen gas at slightly less than atmospheric pressure.

5. STORAGE

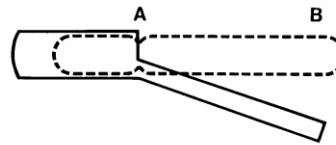
Unopened ampoules should be stored at -20°C.

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

For practical purposes each ampoule contains the same quantity of the above materials. The entire contents of each ampoule should be completely dissolved in an accurately measured amount of solvent. No attempt should be made to weigh out portions of the freeze-dried powder.

For economy in use, it is recommended that the solution, without further dilution, is sub-divided into several small containers and stored at -30°C or below. To maintain full activity of such stored aliquots, it is suggested that the solution of the ampoule contents and its subdivision and freezing by liquid N₂ or a mixture of solid CO₂ and ethanol is done rapidly and that repeated freezing and thawing be avoided. A dilute solution prepared for use in an assay should be kept cool (e.g. 4°C) and should contain not less than 0.1% w/v carrier protein (free of proteolytic enzymes). The material has not been sterilized and the ampoules contain no bacteriostat.

8. PREPARATION OF AMPOULES

(1) ACTH

Some 50 mg of highly purified ACTH (of unknown moisture and salt content) was isolated from human pituitaries by Drs. Scott and Lowry, as described earlier², and generously donated for this reference preparation. The potency of this material (with 95% confidence limits) was estimated to be 227 (147-345) IU/mg, in terms of the IS, by Dr Lowry in an in vitro bioassay³.

(2) DISTRIBUTION INTO AMPOULES

In July 1974, 46.9 mg of the (hygroscopic) ACTH preparation was weighed out and dissolved in 650ml of 0.1M acetic acid containing 0.5% (w/v) mannitol and containing 0.154M sodium chloride (batch AR 114/3); Lister Institute, Elstree). Equal volumes (0.5ml) of this solution were distributed into ampoules and the ampoule contents freeze-dried, secondarily desiccated and sealed under nitrogen as earlier described^{4,5}.

The batch consisted of 1058 ampoules. The mean weight of filling solution in each of 28 tared ampoules was 0.510 g with a range of 4.9% of the mean.

9. ASSESSMENT OF AMPOULE CONTENTS

(1) PHYSICO-CHEMICAL ANALYSES

The nominal content of 11.6 µg of ACTH per ampoule given in an earlier memorandum was the mean of 2 estimates of 13.0 and 10.2 µg. For each estimate, the ACTH of the pooled contents of 12 ampoules of 74/555 was separated from carrier albumin, by chromatography in 0.5M-acetic acid on a column of Biogel P6 (60 x 1cm) (which had been saturated with porcine and then human ACTH to minimize losses due to adsorption), and then determined from its absorbance at 280nm (assuming that A^{1%}_{1cm} = 17.7⁶).

Subsequently, using different methods, the content of ACTH in 74/555 was found to be considerably higher.



(a) ACTH of the pooled contents of 10 ampoules was estimated by amino acid analysis, after its isolation by chromatography on Biogel P6. By this method each ampoule was found to contain 27 µg of ACTH, or 35-37 µg when corrections were made for losses, estimated by immunoassay, to have occurred during chromatography on Biogel P6.

(b) The ACTH content of 74/555 was also estimated by isoelectric focusing the ampoule contents in a polyacrylamide gel^{7,8}, which separated ACTH from albumin and permitted duplicate estimates from the contents of a single ampoule. By this method, the ACTH preparation was found to be more than 90% pure and the content per ampoule of the main component was estimated to be 23.5µg (the mean estimate from 3 ampoules), in terms of a preparation of purified porcine ACTH.

(2) BIOLOGICAL ASSAYS

Assay Method	No. of assays	ACTH content of 74/555 as IU/ampoule in terms of the IS	
		Weighted geometric mean	95% confidence limits
Adrenal ascorbate depletion ⁸ . (s.c administration)	3	3.20	2.61-3.92
Plasma corticosterone increase ⁸ (i.v administration)	3	6.16	5.03-7.55
Adrenal corticosterone increase ⁹ . (i.v administration)	2	6.13	4.88-8.38
Adrenal corticosterone production ⁸ (<i>In vitro</i>)	5*	6.54	6.18-6.93

*Three further assays were excluded because of significant ($P < 0.05$) deviation from linearity of, or parallelism between, the slopes of the log dose-response curves of test and standard. Another assay which gave a potency estimate of 2.63 IU/ampoule was also excluded.

The estimated potency of 74/555 in terms of the IS differed according to the assay method, especially between assays using intravenous or subcutaneous administration; reminiscent of earlier experience with differing corticotrophin preparations¹. The log potency estimates obtained by the two i.v assay methods were homogeneous and combined to give a weighted geometric mean potency (with 95% confidence limits) of 6.15 (5.19-7.29) IU/ampoule. (One estimate of 4.90 IU/ampoule by the intravenous method of Lipscomb and Nelson¹⁰ was omitted from this computation).

10. STABILITY

In the absence of stability data, users should assume the reference preparation to exhibit the potency as described at establishment

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.

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 the appropriate NIBSC scientist.

In addition, once reconstituted, diluted or aliquoted, 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.

11. REFERENCES

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12. ACKNOWLEDGEMENTS

Grateful acknowledgments are due to Drs. P. J Lowry and A. P Scott (The Royal Hospital of St. Bartholomew, London) for isolating, characterizing and providing the highly purified corticotrophin ; Dr P. J Campbell (NIBSC) for ampouling; Drs D. H Calam, E.A Johnson, Mrs H. Thomas and Mr R.J Tiplady (NIBSC) and Dr P. J. Lowry for peptide analyses; Professor G. M Besser (The Royal Hospital of St. Bartholomew) and Dr J.G Ratcliffe (The Royal Infirmary Glasgow) for immunoassays.

13. 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

14. 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

15. 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.

16. 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: Yes	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 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.	

17. 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.

18. 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: 6mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No