



**WHO International Standard  
Growth Hormone, Human, Pituitary  
NIBSC code: 80/505  
Instructions for use  
(Version 3.0, Dated 04/12/2007)**

**1. INTENDED USE**

This International Standard consists of a batch of ampoules (coded 80/505) containing highly purified pituitary human growth hormone (hGH). Initially established as the International Standard for hGH for bioassay, this preparation (coded 80/505), has now been established by the Expert Committee on Biological Standardization of WHO at its 38th Meeting (December 1987), as the 1st I.S. for hGH without the designation 'for bioassay' and with unchanged unitage. It replaces the International Reference Preparation of hGH for Immunoassay (coded 66/217), which is now exhausted.

**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. However, as with all materials of human origin, the preparation cannot be assumed to be free from infectious agents. The container and its contents should be used and discarded according to your own laboratory procedures. Such procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening the container to avoid cuts.

**3. UNITAGE**

By definition 4.4 International Units per ampoule.

The International unit of 80/505 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 80/505 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.24%.

**4. CONTENTS**

Country of origin of biological material: United Kingdom.

Each ampoule contains the freeze-dried residue of 1.0ml of a solution which contained:

hGH extract	approx	1.70 mg
Glycine	"	20.0 mg
Mannitol	"	2.0 mg
Lactose	"	2.0 mg
Sodium bicarbonate	"	2.0 mg
pH 7.3		

**5. STORAGE**

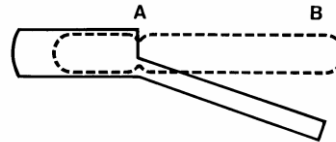
Unopened ampoules should be stored at -20°C.

**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 all practical purposes each ampoule contains the same quantity of the above materials. The entire contents of each ampoule should be completely dissolved in a known volume of buffer (with carrier protein, where extensive dilution is required). No attempt should be made to weigh out portions of the freeze-dried powder.

**8. PREPARATION OF AMPOULES**

(a) HGH

Approximately 6.4g of highly purified hGH was isolated from frozen glands as described by Lumley Jones *et al* (1979) and kindly donated to the World Health Organization. It was further purified by gel filtration (on Ultrogel ACA 44) by Dr. A. Phillips (Wellcome Research Laboratories, Kent).

(b) DISTRIBUTION INTO AMPOULES

6.4gm of hGH (batch no. R/FP/SL5) were dissolved in a sterile pyrogen-free solution containing 2% glycine, 0.2% mannitol, 0.2% lactose and 0.25% sodium bicarbonate, pH 7.3, to a final concentration of 1.735mg hGH/ml of solution. This was filtered through a Unipore polycarbonate membrane of 3µm mean pore diameter and equal volumes of the solution were distributed into ampoules. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen, according to the procedure used for international biological standards (Annex 4, 29th ECBS Report). The batch consists of 3,900 ampoules. The mean weight of filling solution dispensed into weighed ampoules at intervals throughout the fill was 1.003g (± 0.2%).

(c) EVALUATION OF AMPOULE CONTENTS

(i) Twenty-two laboratories in 10 countries took part in the international collaborative study to evaluate the Standard, (Bangham, Gaines Das and Schulster, 1985). The hGH was estimated in bioassays against the International Standard for Growth Hormone, Bovine (established 1955) hitherto used for bioassays of hGH preparations. Estimates by the body weight gain assay in hypophysectomized rats and in dwarf (Snell) mice gave a geometric mean value of 4.30 u/ampoule (95% fiducial interval 3.83-4.81 u/ampoule); by the tibial epiphyseal width increase assay, a geometric mean value of 3.26 u/ampoule (2.57-4.14 u/ampoule). When assayed against the International Reference Preparation of hGH for Immunoassay (coded 66/217) the mean estimate was 4.80 u/ampoule in radioreceptor assays (4.60-5.01 u/ampoule); and in various radioimmunoassays the mean estimate was 5.26 u/ampoule (4.47-6.19 u/ampoule).

(ii) Analysis on polyacrylamide gel, pH 7.8 indicated a predominant band of 22K hGH, about 5% 20K and small amounts of 'slow', dimer and desamido hGH, as well as trace amounts of several other proteins.

(iii) Estimates of other substances present include:



Arginine vasopressin	<8 miu/amp	(bioassay)
Oxytocin	0.8 ng/amp	"
ACTH	<0.3x10 <sup>-3</sup> iu/amp	(adrenal cell bioassay)
TSH	0.2 miu/amp	(immunoassay)
LH	<11 iu/amp	"
FSH	<5 iu/amp	"
Prolactin	0.16 iu/amp	"

On the basis of the results of the collaborative study and with the agreement of the participants, the Expert Committee on Biological Standardization of WHO established at its 33rd meeting (September 1982) the ampouled preparation coded 80/505 as the (first) International Standard for Growth Hormone, Human, for Bioassay and, on the basis of the assay data, assigned to each ampoule 4.4 International Units of growth hormone activity.

At the 38th meeting (1987), the preparation was established as the 1st International Standard for Growth Hormone, Human, with the same unitage (4.4 IU) per ampoule.

### 9. 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 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. Unopened ampoules should be stored on receipt as indicated on the label. 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.

### 10. REFERENCES

Bangham DR, Gaines Das RE & Schulster D (1985). Molecular & Cellular Endocrinology, **42**, 282-296.  
Lumley-Jones R, Benker G, Salacinski PR, Lloyd TJ & Lowry PJ (1979). J. Endocrinol **82**, 77-86.  
Annex 4, 29th Report WHO Expert Committee on Biological Standardization. WHO Tech. Rep. Ser. No. 626, 1978.

### 11. ACKNOWLEDGEMENTS

Acknowledgements are due to the U.K. Pituitary Collection for providing glands used in this study; Dr PJ Lowry, Dr A Phillips and colleagues for the extraction and purification of the hGH; and to Dr PJ Campbell for ampouling.

### 12. 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](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](http://www.nibsc.org/terms_and_conditions.aspx)

### 13. CUSTOMER FEEDBACK

National Institute for Biological Standards and Control,  
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, [nibsc.org](http://nibsc.org)  
WHO International Laboratory for Biological Standards,  
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### 14. 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. 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: Freezed powder	dried 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.	

### 16. 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](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.



#### 17. INFORMATION FOR CUSTOMS USE ONLY

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 28mg
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> 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\\_biologicalstandardsrev2004.pdf](http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biologicalstandardsrev2004.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.