WHO Reference Reagent
Human Chorionic Gonadotrophin,
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified
Beta Subunit (Purified)
NIBSC code: 99/650
Instructions for use
(Version 4.0, Dated 24/07/2013)

1. INTENDED USE
This consists of a batch of lyophilised ampoules containing the beta subunit of human chorionic gonadotrophin, purified to remove other forms of human chorionic gonadotrophin, including nicked forms of hCG and its subunits. The preparation was established as the first WHO Reference Reagent at the 2001 meeting of the Expert Committee on Biological Standardization.
In making this recommendation the committee noted that the preparation 99/650 is not intended to replace the existing International Standard for hCG-beta as the primary calibrant for immunoassays. The Reference Reagent 99/650 is intended for use in investigating and characterizing the specificity of existing hCG and hCG-β assays.

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
0.88nmol/ampoule, by definition
The nmol value of 99/650 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 99/650 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.09%.

4. CONTENTS
Country of origin of biological material: United Kingdom.
Lyophilised residue containing approximately 1.0 nmol purified human chorionic gonadotrophin-β, 2 mg human serum albumin, phosphate buffer salts (50mM pH7.4)

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. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL
For practical purposes each ampoule contains the same quantity of hCG-β. The entire content of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. No attempt should be made to weigh out portions of the freeze-dried powder. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

COLLABORATIVE STUDY
The preparation was evaluated in a collaborative study carried out in 11 laboratories in 5 countries, organised with the following aims:
1) to determine the suitability of the ampouled preparation to serve as a WHO International Standard for purified hCG-β
2) to determine the content of hCG-β in nmol per ampoule by a combination of amino-acid analysis and immunoassay recovery estimates
3) to compare the ampouled preparation with existing International Standards for hCG-β in the various immunoassay systems in use, and to obtain estimates of the ampoule contents in International Units
4) to obtain suitable information confirming the predicted stability of the candidate standard
The overall estimate of ampoule content by amino-acid analysis was 0.88nmol/ampoule. Immunoassay estimates in terms of the International Standard for hCG-β were in the range 20-9430 IU/ampoule and were markedly dependent on the assay system specificity, as would be predicted. The preparation exhibited no significant loss of activity upon storage at elevated temperatures, and is considered stable. Cross-contamination estimates, determined using immunoassays of known specificity, were:

<table>
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<tr>
<th>Component</th>
<th>Estimate</th>
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<tbody>
<tr>
<td>hCG/Nicked hCG</td>
<td>0.725%</td>
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<tr>
<td>hCG beta core fragment</td>
<td>0.139%</td>
</tr>
<tr>
<td>hCG-alpha</td>
<td>0.16%</td>
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8. STABILITY
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.

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.

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

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

9. 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

10. 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

11. 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.

12. MATERIAL SAFETY SHEET

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
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<tbody>
<tr>
<td>Physical appearance: White lyophilised powder</td>
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<tr>
<td>Stable: Yes</td>
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<tr>
<td>Hygroscopic: Yes</td>
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<tr>
<td>Flammable: No</td>
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<tr>
<td>Other (specify): Can react with oxidising materials. Avoid contact with acids and alkalis.</td>
</tr>
</tbody>
</table>

Toxicological properties

- Effects of inhalation: No adverse effects have been reported for this material.
- Effects of ingestion: No adverse effects have been reported for this material.
- Effects of skin absorption: No adverse effects have been reported for this material.

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.

13. 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. 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: 2 mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. 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_biol_estandardsrev2004.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.