Influenza Reagent
Influenza virus infectious NIB-50
NIBSC code: 07/358
Instructions for use
(Version 1.0, Dated 17/03/2008)

1. INTENDED USE
Reagent 07/358 is prepared from NIB-50 (A/Uruguay/76/2007 (H3N2) x
A/PR/8/34 (H1N1) ) which was processed for freeze drying in 250μl
volumes as described by Campbell, P.J. Journal of Biological
Standardisation, 1974, 2,249-267. The known passage history of NIB- 50
is attached

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
No unitage is assigned to this material

4. CONTENTS
Country of origin of biological material: United Kingdom.
Each ampoule contains 250μl (nominal) of infectious influenza virus as
allantoic fluid from SPF embryonated hen’s eggs.

5. STORAGE
Store in the dark at -20C or below
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. Various types of
ampoule breaker are available commercially. To open the ampoule,
tap the ampoule gently to collect material at the bottom (labelled) end
and follow manufactures instructions provided with the ampoule
breaker.

7. USE OF MATERIAL
Reconstitute the contents of one ampoule of reagent with 250μl of sterile
distilled water. Leave for a minimum of 5 minutes before use to allow for
complete solution of freeze-dried material. A range of dilutions (e.g.10^3 to
10^-5) should be made in a suitable medium for initial cultivation.

8. STABILITY
Reference Materials should be stored on receipt as indicated on the
label.

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

9. REFERENCES
NA

10. ACKNOWLEDGEMENTS
NA

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Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
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<tbody>
<tr>
<td>Physical appearance:</td>
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<tr>
<td>Stable:</td>
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<tr>
<td>Hygroscopic:</td>
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<tr>
<td>Flammable:</td>
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<tr>
<td>Other (specify):</td>
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</table>

<table>
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<tr>
<th>Toxicological properties</th>
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<tbody>
<tr>
<td>Effects of inhalation:</td>
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<tr>
<td>Effects of ingestion:</td>
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<tr>
<td>Effects of skin absorption:</td>
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</tbody>
</table>

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 virucidal agent. Rinse area with an
appropriate virucidal agent followed by water.
Absorbent materials used to treat spillage should be treated as
biologically hazardous 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
request by the Recipient) ("Conditions") apply to the exclusion of all other
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**: NA

**Toxicity Statement**: Non-toxic

<table>
<thead>
<tr>
<th>Passage</th>
<th>Lot</th>
<th>Laboratory</th>
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<tbody>
<tr>
<td>E : E4</td>
<td></td>
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<tr>
<td>E5</td>
<td>29650</td>
<td>NIBSC, Hertfordshire, UK</td>
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**Veterinary certificate or other statement if applicable**.

**Passage history of NIB-50 (post mixed infection)**
Derivation of NIB-50

A/Uruguay/716/2007 (H3N2)-like High Growth Reassortant

Strain: A/Uruguay/716/2007 (H3N2)
Received from CDC #2007731384, SpfCKE3
Passage undertaken at NIBSC #29460, SpfCKE4

Mixed Infection: A/Uruguay/716/2007 (10^2) x A/PR/8/34 (H1N1)(10^3)
HA Titre: 2560

1st Antiserum passage: Inoculum 10^3 with A/PR/8/34 antiserum
HA Titre: 2048

2nd Antiserum passage: Inoculum 10^3 with A/PR/8/34 antiserum
HA Titre: 640

3rd Antiserum passage: Inoculum 10^4 with A/PR/8/34 antiserum
HA Titre: 1280

4th passage: Inoculum 10^9
HA Titre: 5120

5th passage (seed material): Inoculum 10^9
HA Titre: 1280
Lot 29650

Total number of passages since mixed infection=ES.
SPF eggs were used for all passages.

RT-PCR/RFLP analysis indicates that NIB-50 has HA and NA genes from A/Uruguay/716/2007 and NP, NS, PB1, PB2, PA and M genes from A/PR/8/34 making it a 6:2 reassortant.
The HI data below was provided by the WHO Collaborating Center, NIMR, London, UK. Based on that data they concluded that NIB-50 is similar to wild type A/Uruguay/716/2007(H3N2)

Antigenic analyses of influenza A H3N2 viruses (26/02/08)

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