



**Influenza Reagent**  
**Influenza antiserum A/Cambodia/R0405050/2007 (H5N1)**  
**NIBSC code: 08/210**  
**Instructions for use**  
**(Version 3.0, Dated 25/08/2011)**

#### 1. INTENDED USE

Influenza antiserum reagent 08/210 is prepared in sheep for single radial diffusion assay of A/Cambodia/R0405050/2007 antigens. An appropriate NIBSC reagent should be included in each assay

#### 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.  
The antiserum reagent was prepared in sheep SH489 and SH494 to the purified HA of NIBRG-86 (a reassortant prepared by reverse genetics with HA (modified to remove the multi basic cleavage site) and NA from A/Cambodia/R0405050/2007). The HA antigen was extracted from purified virus by treatment with bromelain and purified by sedimentation on sucrose gradients (Brand, CN and Skehel, JJ, Nature, New Biology, 1972, 238, 145-147).

The immunisation schedule for sheep SH489 was as follows: One dose of approximately 50 micrograms of HA with Freund's Complete Adjuvant (FCA) was given intramuscularly, followed two weeks later with a 10 microgram dose of HA with Freund's Incomplete Adjuvant (FIA), four further 10 microgram doses of HA with FIA were given at weekly intervals. Seven weeks after the initial immunisation, serum was collected and sodium azide (0.05% w/v) was added. The immunisation schedule for sheep SH494 was as follows: One dose of approximately 50 micrograms of HA with FCA was given intramuscularly, followed two weeks later with a 10 microgram dose of HA with FIA, three further 10 microgram doses of HA with FIA were given at weekly intervals, followed by a further seven 20 microgram doses of HA with FIA, also given at weekly intervals. Thirteen weeks after the initial immunisation, serum was collected and sodium azide (0.05% w/v) was added.

The serum from SH489 was treated by maintenance of pH5.49 for 40 minutes followed by restoration of the original pH. The serum from SH494 was treated by maintenance of pH5.49 for 40 minutes followed by restoration of the original pH. The serum from SH489 and SH494 was then pooled and diluted 1:2 with PBS buffer containing sodium azide (0.05% w/v) and filled into vials in 2ml volumes.

#### 5. STORAGE

+2-8°C

**Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.**

#### 6. DIRECTIONS FOR OPENING

Vials have a 'flip-up' circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and

withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

#### 7. USE OF MATERIAL

For the assay of antigens containing 20-50 micrograms of HA activity in 1ml, approximately 20µl of the undiluted Reagent should be added to 1ml agarose. It may be necessary to change the antiserum concentrations according to local laboratory conditions.

Antiserum reagent 08/210 should be used according to the method described by Wood JM, Schild GC, Newman RW and Seagroatt VA. Journal of Biological Standardisation, 1977, 5, 237-247.

#### 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label, please also see attached storage information sheet.

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

#### 9. REFERENCES

N/A

#### 10. ACKNOWLEDGEMENTS

This reagent was produced on behalf of the FLUSECURE project with funding from the EU.

#### 11. FURTHER INFORMATION

Further information can be obtained as follows;

This material: [enquiries@nibsc.org](mailto: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)

#### 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](mailto: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

<b>Physical and Chemical properties</b>	
Physical appearance: straw coloured liquid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains sheep serum and sodium azide	
<b>Toxicological properties</b>	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
<b>Suggested First Aid</b>	
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.
<b>Action on Spillage and Method of Disposal</b>	
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.	

#### 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](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.

#### 16. 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> 2g
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable. <b>Attached:</b> Yes SH489 and SH494 Plus FMD inactivation Certificates for SH489 and SH494. Plus Storage Information sheet.



## STORAGE OF REAGENT 08/210

NIBSC has prepared a number of reagents for single radial diffusion assay of influenza subtypes of pandemic potential.

Since it is not known when these reagents may be required, it is desirable that they have an indefinite shelf life and they are stored at NIBSC in colder conditions than reagents prepared for the assay of epidemic strains. Therefore the recommended storage temperature marked on the label for reagent 08/210 is  $-20^{\circ}\text{C}$ .

However it is assumed that a customer ordering this reagent, will use it within a short period similar to that for a conventional reagent. Consequently, this reagent is not normally shipped frozen and **the recommended storage temperature is  $+4^{\circ}\text{C}$** .



Blanch Lane  
South Mimms  
Potters Bar  
Hertfordshire  
EN6 3QG  
Tel: +44 (0) 1707 641000  
Fax: +44 (0)1707 646730  
Email: [enquiries@nibsc.ac.uk](mailto:enquiries@nibsc.ac.uk)  
<http://www.nibsc.ac.uk>

#### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a Sheep with ear tag number: UK 281038 5383 [Virology no SH 489], which has been used in the production of blood antiserum between 9<sup>th</sup> July 2008 and 26<sup>th</sup> August 2008. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.

 26<sup>th</sup> August 2008

Kath Hardcastle BVetMed MRCVS  
Named Veterinary surgeon  
Royal Veterinary College  
Royal college Street  
London  
NW1 0TU



Blanche Lane  
South Mimms  
Potters Bar  
Hertfordshire EN6 3QG  
United Kingdom

Tel: +44 (0)1707 641000  
Fax: +44 (0)1707 641050  
Email: [enquiries@nibsc.ac.uk](mailto:enquiries@nibsc.ac.uk)  
Web: <http://www.nibsc.ac.uk>

### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 281 038 5383 [Virology no. SH489] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. The treatment method used was maintenance of pH 5.5 or lower for a minimum of 40 minutes.

Dr Philip Minor  
Deputy Director  
National Institute for Biological Standards and Control



National Institute for Biological Standards and Control





Blanche Lane  
South Mimms  
Potters Bar  
Hertfordshire EN6 3QG  
United Kingdom

Tel: +44 (0)1707 641000  
Fax: +44 (0)1707 641050  
Email: [enquiries@nibsc.ac.uk](mailto:enquiries@nibsc.ac.uk)  
Web: <http://www.nibsc.ac.uk>

### Foot and Mouth Disease Virus Inactivation Certificate

This is to certify that serum collected from Sheep no. UK 181 445 2199 [Virology no. SH494] has been treated by an APHIS approved method for inactivation of Foot and Mouth Disease Virus. The treatment method used was maintenance of pH 5.5 or lower for a minimum of 40 minutes.

Dr Philip Minor  
Deputy Director  
National Institute for Biological Standards and Control



National Institute for Biological Standards and Control





**RVC**

Royal Veterinary College  
University of London

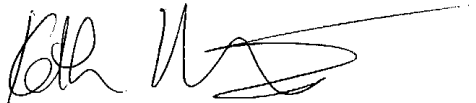
**Kath Hardcastle**  
**Named Veterinary Surgeon**  
**Royal Veterinary College**  
**Royal College Street**  
**LONDON**  
**NW1 0TU**

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### VETERINARY CERTIFICATE OF ANIMAL HEALTH

This is to certify that I have today examined a Sheep with ear tag number: UK 181445 2199 [Virology no. SH494], which has been used in the production of blood antiserum between 6<sup>th</sup> August 2008 and 5<sup>th</sup> November 2008. Both the ear tag number and the animals' record show that it is of UK origin.

This animal was a breeding Ewe which became surplus to requirements. In my opinion at the time of clinical examination, the ewe was in good health and showed no clinical signs of infectious disease.



Kath Hardcastle BVetMed Cert LAS MRCVS  
Named Veterinary Surgeon

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**Kath Hardcastle BVetMed Cert LAS MRCVS**  
**Office: 020 7468 5333 Fax: 020 7468 5378 Mobile: 07809199998**  
**Email: <khardcastle@rvc.ac.uk>**