



**Influenza Reagent
Influenza Virus Infectious IVR-224 (H3N2)
NIBSC code: 21/196
Instructions for use
(Version 1.0, Dated 28/07/2021)**

1. INTENDED USE

Reagent 21/196 is prepared from IVR-224 (A/Cambodia/E0826360/2020 (H3N2) x A/PR/8/34) which was processed in 250µl volumes as liquid stock. The derivation and known passage history of IVR-224 (H3N2) 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 vial contains 250µl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen's eggs.

5. STORAGE

Store in the dark at -70°C or below

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

Ready to use

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

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:

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

<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

Physical and Chemical properties	
Physical appearance: Clear liquid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Live influenza virus	
Toxicological properties	
Effects of inhalation:	Likelihood of influenza virus infection
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 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 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

<p>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.</p> <p>Net weight: 0.25g per vial</p>
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<p>Toxicity Statement: Non-toxic</p> <p>Veterinary certificate or other statement if applicable.</p> <p>Attached: No</p>

Passage history of IVR-224 (H3N2)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E1-E5	E5	Unknown	Unknown
E6-E12	E5/E7	Lot 512	Seqirus, Australia
E13	E5/E7/E1	46330	NIBSC, UK

Sterility: No visible contamination was detected in a variety of media (tryptose soya broth, thioglycolate broth, Sabouraud’s broth and blood agar plates) after 14 days incubation.

Sanger sequencing analysis suggests this product may contain a minority population with a non-synonymous mutation in the HA gene that results in a Serine to Phenylalanine change at amino acid 219 (Mature HA numbering).

The HA and NA sequence of this virus is available at GISAID with the accession number EPI_ISL_3084570.



REPORT

PASSAGE HISTORY:

<i>Mixed infection passage:</i> (E5/D1)	A/Cambodia/e0826360/2020 wt virus@10 ⁻³ x A/Puerto Rico/8/34 (H1N1) @10 ⁻³ ↓	HA titre ≥1631 Guinea Pig RBC
<i>1st Antiserum Passage</i> (E5/D2)	Inoculum @ 10 ⁻³ with antiserum to A/Puerto Rico/8/34 (H1N1) ↓	HA titre = 331 Guinea Pig RBC
<i>2nd Antiserum Passage</i> (E5/D3)	Inoculum @ 10 ⁻³ with antiserum to A/Puerto Rico/8/34 (H1N1) ↓	HA titre =37 Guinea Pig RBC
<i>3rd Antiserum/ 1st Limit Dilution **</i> <i>Passage</i> (E5/D4)	Inoculum @ 10 ⁻⁸ ↓	HA titre =72 Guinea Pig RBC
<i>2nd Limit Dilution Passage</i> (E5/D5)	Inoculum @ 10 ⁻¹⁰ ↓	HA titre =106 Guinea Pig RBC
<i>3rd Limit Dilution Passage</i> (E5/D6)	Inoculum @ 10 ⁻⁸ ↓	HA titre =320 Guinea Pig RBC HA titre = ≥1631 Turkey RBC
<i>4th Limit Dilution Passage</i> Lot 512 (E5/D7) IVR-224	Inoculum @ 10 ⁻⁵	HA titre = 80 Guinea Pig RBC HA titre =663 Turkey RBC

** Virus sample diluted to 10⁻³, dilution was mixed with antiserum to A/Puerto Rico/8/34 (H1N1) and incubated for 1 hour at room temperature. Incubated virus/antiserum sample was serially diluted and inoculated into eggs.