



Influenza Reagent
Influenza virus infectious X-347A (H3N2)
NIBSC code: 20/296
Instructions for use
(Version 4.0, Dated 22/03/2021)

1. INTENDED USE

Reagent 20/296 is prepared from X-347A (H3N2) (A/Paris/2554/2019 (H3N2) x A/PR/8/34) which was processed in 250µl volumes as liquid stock. The derivation and known passage history of X-347A (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

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: 0.25g per vial
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Toxicity Statement: Non-toxic Veterinary certificate or other statement if applicable. Attached: No
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Passage history of X-347A (H3N2)

Cumulative number of passages	Passage numbers at each stage	Lot	Laboratory
E1-E4	E4	Unknown	Unknown
E5-E14	E4/E10	E#6447	NYMC, USA
E15	E4/E10/E1	45680	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.

The HA and NA sequence of this virus is available at GISAID with the accession number EPI_ISL_883194.



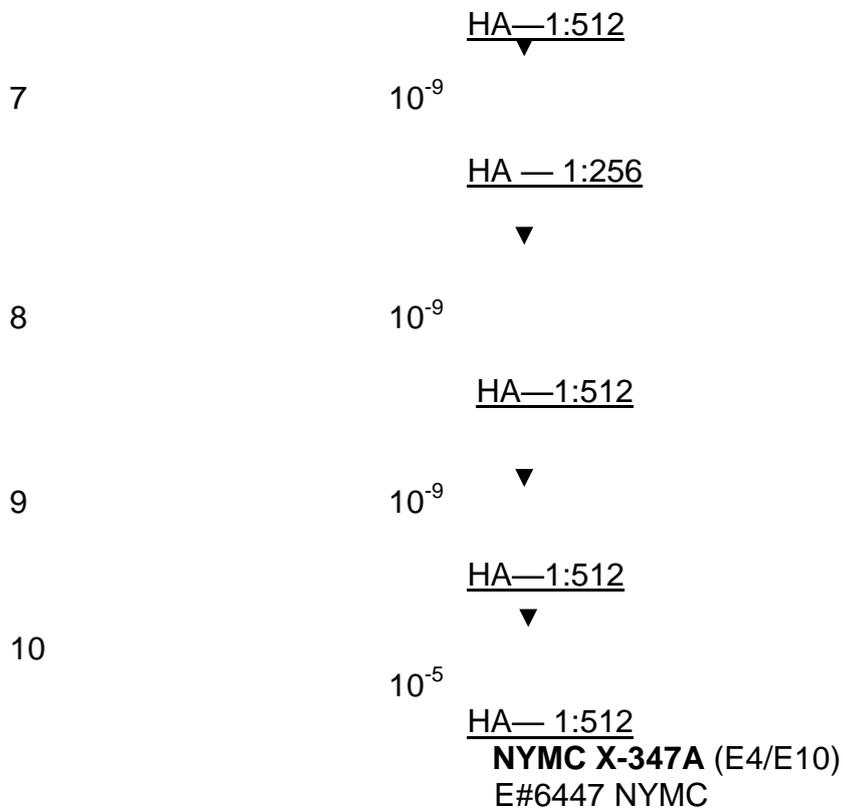
Derivation of NYMC X-347A
A/Paris/2554/2019 (H3N2) with A/PR/8/34
High Yield A H3N2 Reassortant (5:3)
with A/PR/8/34 M, PB2,PA, NS and NP genes and
A/Paris/2554/2019 PB1, HA, and NA genes

Exper. # 4859
 A/Paris/2554/2019
 H3N2
 #201245 E4 (Am2A12)
 GP 128(10⁻⁵) 20/2/2020

Passages at New York Medical College

Passage No.

1	10 ⁻¹	
	HA—1:32	
	▼	
	<u>A/Paris/2554/2019 (H3N2) x A/PR/8/34</u>	
2	10 ⁻¹ + 10 ⁻³	
	HA—1:2048	
	▼	
3	10 ⁻¹	+ A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)
	HA—1:1024	
	▼	
4	10 ⁻¹	+ A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)
	HA-1:256	
	▼	
5	10 ⁻³	+ A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)
	HA—128	
	▼	
6	10 ⁻⁴	



HA Yield by UPLC Analysis ($\mu\text{g HA/ml}$ allantoic fluid)

wt (wild type)	X-347A	Fold Increase
5.8	8.1	1.4

HA and NA, genes were identified as A/Paris/2554/2019 by RT-PCR/RFLP gene analysis. PA, NS and NP genes were identified as A/PR/8/34 by RT-PCR/RFLP analysis.

The PB1 gene was identified as A/Paris/2554/2019 by qPCR analysis. M and PB2 genes were identified as A/PR/8/34 by qPCR analysis.

SPF eggs were used for all reassortant passages.

Virus seed was shown to be sterile. Sterility testing was performed by streaking the sample on blood agar plates and incubating for 48 hours at 37 °C.