

Influenza A H5 Positive Control 1

SUMMARY

Influenza A H5N3 (A/Duck/Singapore-Q/F119-3/97) virus in L6 lysis buffer is issued in 2 x 800 µL volumes.

INTENDED USE

Influenza A H5 positive control is intended for use as a reverse-transcription / PCR (RT-PCR) run control for the real-time or the block-based Influenza A H5 specific RT-PCR assays (NSM VSOP 40 and 41).

CONTENTS

2 x 0.8mL of ready-to-use inactivated virus in
2 x 2mL Sarstedt polypropylene tubes

COMPOSITION

Influenza A H5 Concentration	2.75 X 10 ⁶ PFU/µL
L6 Lysis buffer	Severn Biotech Ltd

MATERIALS REQUIRED BUT NOT PROVIDED

Details of RT-PCR reagents necessary are given in the National Standard Methods VSOP 40 and 41 available from www.hpa-standardmethods.org.uk

WARNINGS AND PRECAUTIONS

This reagent is for *in-vitro* use only.

This virus has no polybasic cleavage site in the HA and is apathogenic. The reagent is inactivated in lysis buffer.

ACKNOWLEDGEMENTS

A/Duck/Singapore-Q/F119-3/97 was kindly provided by the Director of Primary Production, Veterinary Laboratory Branch, Central Veterinary Laboratory Singapore.

Preparation of the control material was carried out by Miss Lynsey Whilding at HPA, Colindale.

Validation of RNA was carried out by Mr Richard Allan and Dr Joanna Ellis, at HPA, Colindale.

DISPOSAL CONSIDERATIONS

It is the responsibility of each user to handle waste and effluents produced according to their type and degree of hazard and to treat and dispose of them in accordance with any applicable regulations.

Treat this reagent as clinical waste and dispose of according to clinical waste policies in place.

PREPARATION

Twenty eggs in total were inoculated, each with 100µL of control virus; 10-fold serial dilutions of virus were prepared in viral transport medium (VTM). Five eggs were inoculated with dilutions of 10⁻⁵-10⁻⁸. All were incubated at 35°C for 3 days (SOP V5408). Allantoic fluid was then harvested and tested in HA assays. HA positive allantoic fluids obtained from 10⁻⁶-10⁻⁸ dilutions (average HA titre 5120) were pooled and aliquotted into L6 lysis buffer, and stored at -80°C immediately.

INSTRUCTIONS FOR USE

1. Use suitable (latex/nitrile) gloves and eye/skin protection
2. Intended use of reagent is as positive control material for real-time or the block-based Influenza A H5 specific RT-PCR assays
3. Avoid multiple freeze thaw cycles as this could degrade the RNA and seriously affect performance in the subsequent RT/PCR reactions

HANDLING AND STORAGE CONDITIONS

- Avoid contact with skin and eyes
- Reagent must be stored at -80°C upon receipt
- 0.8mL of inactivated virus is sufficient for four extractions. Once thawed, the inactivated virus should be divided into 200µL sub-aliquots of one use and stored at -80°C to avoid freeze/thaw cycles.
- When thawed for use, the aliquot should be extracted then, used in an RT or RT-PCR reaction (Resultant cDNA should be stored at -20°C until needed, RNA should be stored at -80 °C).
- Ensure all tubes and diluent H₂O are nuclease-free as contamination of this product may alter product performance

TRANSPORT INFORMATION

Shipping Name:	Diagnostic Specimen
Class/Division	6.2
UN	3373
Packaging Instruction	PI-650

ACCIDENTAL RELEASE MEASURES

In the event of a spill or leakage, wear suitable eye/skin protection. Use absorbent material to soak up spill. Wipe area with appropriate bactericidal/viricidal agent. Rinse area with water.

Treat all absorbent material used to clean up spill as biological hazardous waste.