TechNotes

HyTest

Clinical and Research Area | Infectious Diseases

Influenza Virus Types A and B

Influenza – or flu – is a respiratory illness that is caused by influenza viruses. Influenza viruses type A and type B cause seasonal epidemics in human beings on an annual basis. Furthermore, influenza A is also responsible for the pandemics that periodically appear, the most recent one being that which was caused by a H1N1 strain in 2009.

The illnesses range from mild to severe. According to WHO, the annual epidemics result in several million cases of severe illnesses and approximately 250,000 to 500,000 deaths per year worldwide. Severe cases and deaths mostly occur among the people in high-risk groups, e.g. young children, pregnant women, people aged over 65 years and those who suffer from certain medical conditions.

Influenza diagnostic tests are based on various technologies from viral isolation to immunodiagnostic and molecular diagnostic methods. Immunodiagnostic tests, especially rapid influenza diagnostic tests (RIDTs) that utilize monoclonal antibodies (MAbs) are often the test of choice due to their ease-of-use and low cost, although the sensitivities of the tests do vary significantly.

Biochemistry of influenza viruses type A and type B

The influenza viruses type A and type B are negative-sense single-stranded RNA viruses that belong to the family of *Orthomyxoviridae*. These enveloped viruses are usually spherical and 30-100 nm in diameter. The ssRNA is found in ribonucleoparticle complexes that are associated with RNA-dependent polymerase and nucleoprotein (NP). NP is a structural protein that is one of the main determinants of the virus type (A, B or C). Two other important antigens are haemagglutinin (HA) and neuraminidase (NA). Both of these are glycoproteins and they are found on the surface of the virus.

The influenza A viruses are divided into subtypes based on the variations in the HA and NA proteins. There are 18 known types of hemagglutinin and 11 known types of neuraminidase. H1N1 and H3N2 strains are currently circulating as seasonal influenza A viruses.

The influenza B viruses are not divided into subtypes. They are instead named after the areas where they were first identified.

Reagents for the development of immunoassays for the detection of influenza viruses

We provide a broad selection of MAbs with different specificities that enable the detection of influenza A and influenza B from clinical samples. In addition, we provide several strains of inactivated viruses as antigens.

Influenza A monoclonal antibodies

We provide several highly sensitive and specific monoclonal antibodies for the detection of the influenza A virus. MAbs are suitable for common immunoassays such as direct or indirect ELISA, sandwich immunoassays and Western blotting. The antibodies are specific to either different hemagglutinin proteins or influenza A nucleoprotein and can be used to detect these antigens from different biological samples such as nasal aspirates and swabs, cell lysates etc. As MAbs do not have cross-reactivity to the influenza B virus they can be used for differentiation between influenza A and influenza B.

Antibodies specific to influenza A NP (Cat.# 3IN5)

We provide five different MAbs that are specific to influenza type A nucleoprotein. The antibodies were raised against a H1N1 strain. All of the MAbs detect NP with high specificity and do not cross-react with influenza B nucleoprotein.

Anti-NP MAbs equally detect different strains of influenza A in ELISA. An example of a titration curve with the MAb InA108 is provided in Figure 1.

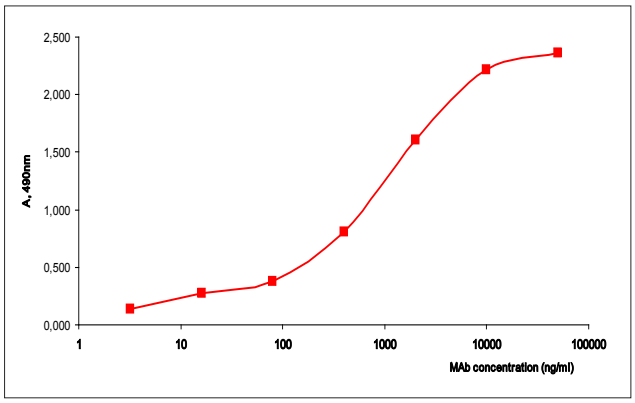

Figure 1. Titration curve of the MAb InA108 that is specific to NP of the influenza A virus in indirect ELISA. Antigen: Influenza A/New Caledonia/20/99 (H1N1); 0.2 µg/well.

Quantitative NP sandwich immunoassays. We tested all MAbs as capture and detection antibodies in sandwich immunoassays. All of the pairs detect nucleoprotein of different influenza A strains. The recommended pairs InA108–InA245 and InA180–InA245 are able to detect the nucleoprotein equally in strains H1N1 and H3N2. The calibration curve for InA108–InA245 is provided in Figure 2.

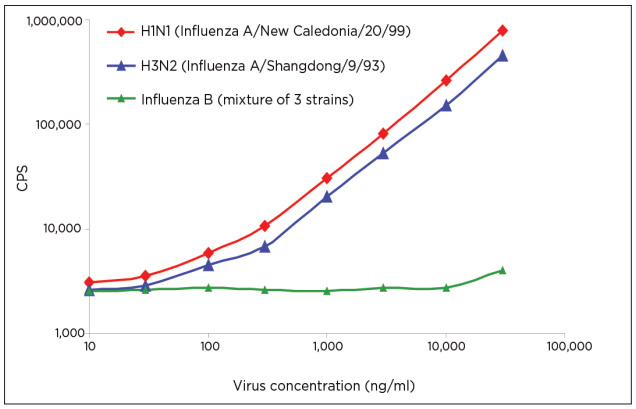

Figure 2. A calibration curve for the influenza A sandwich fluoroimmunoassay using the MAb pair InA108-InA245 that is specific to the nucleoprotein of influenza A (Cat.# 3IN5). The antibody pair recognizes different influenza type A strains but does not detect influenza B strains.

MAb F8. Based on our studies, the MAb F8 recognizes an epitope that can be found in nucleoproteins of influenza type A with different antigenic structure and species origin. We investigated 25 strains of human and avian influenza virus A that had been isolated during different epidemics between 1934 and 1993 and a specific reaction was observed in all of the cases. In addition, we investigated 265 samples of nasal washings from children during influenza outbreaks using direct immunofluorescence. Sensitivity and specificity of the influenza virus A detection was 60% and 98.2% respectively.

The titration curve of MAb F8 is shown in Figure 3.

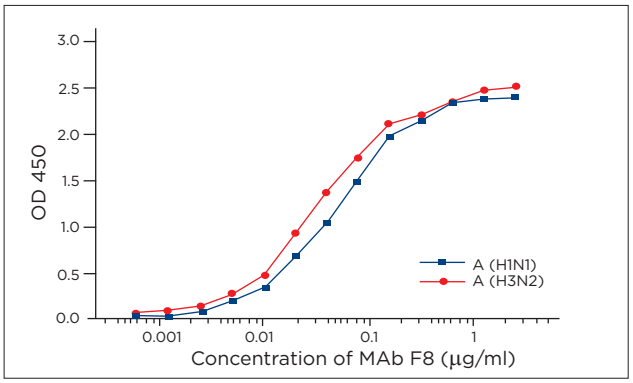

Figure 3. Specific activity of the MAb F8 in ELISA with the purified virus antigens A H1N1 and H3N2.

NP immunodetection in Western blotting. The MAbs InA108 and InA245 can be used for the detection of nucleoprotein in Western Blotting after SDS-PAGE in reducing conditions. The detection of two different influenza A strains with the MAb InA108 is shown in Figure 4.

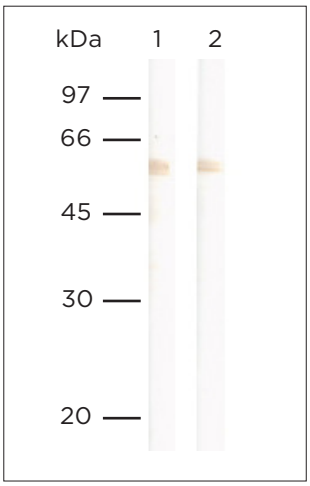

Figure 4. Immunodetection of the influenza A viruses using anti-NP monoclonal antibody InA108 in Western blotting after SDS-PAGE in reducing conditions. Antigens 1 µg/well, MAb InA108 5 µg/ml. Anti-mouse IgG conjugated with HRP was used as the secondary antibody. Lane 1 – Influenza A/NewCaledonia/20/99 (H1N1). Lane 2 – Influenza A/Shangdong/9/93 (H3N2).

Antibodies specific to influenza A H1 (Cat.# 3AH1 and 3IH4)

We provide five MAbs that are specific to hemagglutinin H1. Four antibodies (Cat.# 3AH1) were raised against a purified influenza A strain A/New Caledonia/20/99 (H1N1). One antibody (Cat.# 3IH4) was raised against an avian influenza virus strain A (H1N1).

MAbs that are specific to H1 are able to detect a H1N1 strain in direct and indirect ELISA but are unable to detect a H3N2 strain. Titration curves of the MAb InA4 with two different influenza A strains are provided in Figure 5.

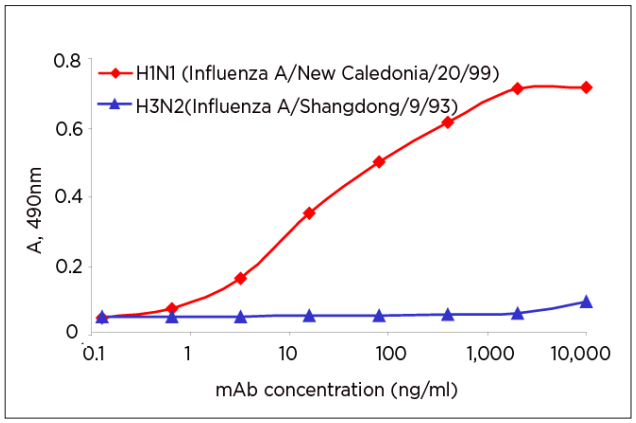

Figure 5. Titration curves of the MAb InA4 that is specific to hemagglutinin H1.

Quantitative H1 sandwich immunoassay. We tested all of the MAbs as capture and detection antibodies in sandwich type fluoroimmunoassays. The pairs InA4–InA134 and InA97–InA134 were able to detect a purified H1N1 strain with high specificity and sensitivity. Both pairs were able to detect the virus and recombinant hemagglutinin H1 and could be used in immunoassays that are specific to different H1-strains of influenza A. A calibration curve for InA97–InA134 is shown in Figure 6.

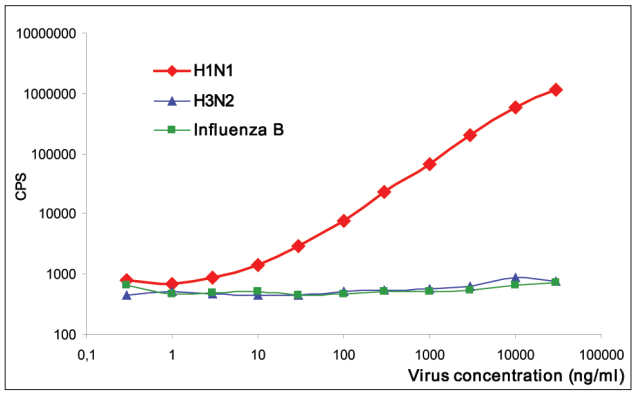

Figure 6. A calibration curve for influenza A sandwich fluoroimmunoassay using anti-haemagglutinin H1 antibodies.
Capture: MAb Capture MAb: InA97 (1 µg/well)
Detection MAb: Eu^{3+} labelled InA134 (0.2 µg/well)
Incubation time: 45 min
H1N1: Influenza A/New Caledonia/20/99
H3N2: Influenza A/Shangdong/9/93
Influenza B: mixture of Influenza B strains B/Qingdao/102/91, B/Tokio/53/99 and B/Victoria/504/00.

MAb C102. This MAb (under Cat.# 3IH4) was obtained by using an avian influenza virus strain A (H1N1) as the immunogen. It is directed against a relatively conservative epitope of H1. The MAb C102 recognizes H1 from human and avian influenza viruses but it does not cross-react with H3 or other hemagglutinins. The MAb C102 could be used for the subtype differentiation of isolates. Meanwhile, the MAb C102 is also suitable for immunocytochemistry, haemagglutinin inhibition, ELISA and immunofluorescence studies. ELISA with the purified virus is provided in Figure 7.

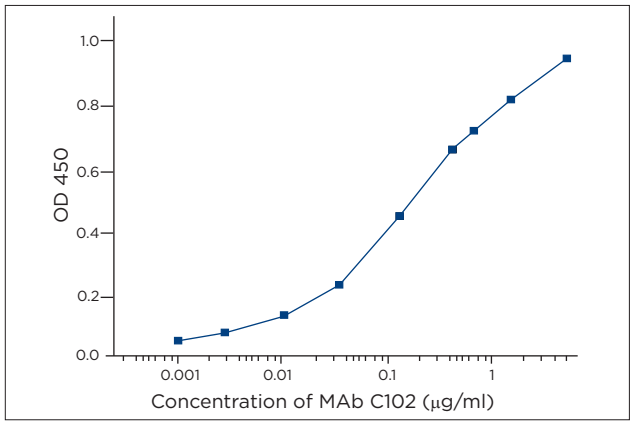

Figure 7. Specific activity of the MAb C102 in ELISA with the purified influenza A virus H1N1.

H1 immunodetection in Western blotting. Anti-H1 antibodies (Cat.# 3AH1) have been tested in Western blotting. All MAbs are suitable for the detection of H1 strains in Western blotting after SDS-PAGE in reducing conditions. An example using the MAb InA4 is shown in Figure 8.

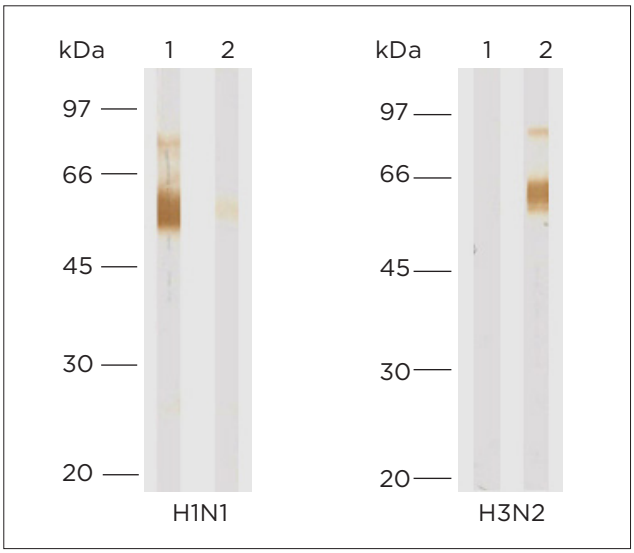

Figure 8. Immunodetection of the influenza A viruses using anti-HA1 and anti-HA3 MAbs in Western blotting after SDS-PAGE in reducing conditions. Strain H1N1: Influenza A/New Caledonia/20/99, strain H3N2: Influenza A/Shangdong/9/93, 1 µg/well. Lane 1 – InA4 (anti-H1), lane 2 – InA246 (anti-H3), 5 µg/ml. Anti-mouse IgG conjugated with HRP was used as the secondary antibody.

Antibodies specific to influenza A H3 (Cat.# 3HG3)

We provide two MAbs that are specific to hemagglutinin H3. The antibodies were raised against the purified influenza A strain A/Shangdong/9/93 (H3N2).

MAbs that are specific to H3 are able to detect a H3N2 strain in direct and indirect ELISA but do not detect a H1N1 strain. Titration curves of the MAb InA246 with two different influenza A strains are provided in Figure 9.

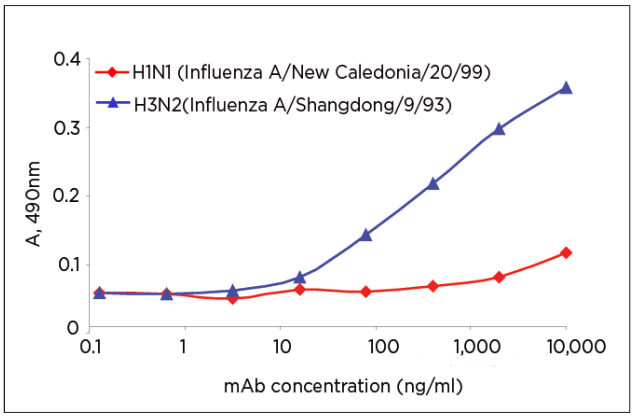

Figure 9. Titration curves of the MAb InA246 that is specific to hemagglutinin H3.

H3 immunodetection in Western blotting. Both of the MAbs detect influenza A H3 strains in Western blotting after SDS-PAGE in reducing conditions. An example using the MAb InA246 is shown in Figure 8.

Antibodies specific to influenza A H5 (Cat.# 3H5N)

Avian influenza viruses – which usually refer to the influenza A viruses – occur naturally in birds and cause avian influenza infection. It is only strains with hemagglutinin H5 and H7 subtypes that can cause highly pathogenic avian influenza which is extremely contagious and rapidly fatal in susceptible avian species. When highly pathogenic influenza H5 viruses cause outbreaks, the mortality rate among poultry is usually between 90 % - 100 %.

In addition to birds, H5 viruses can cause severe infections in humans.

We provide seven different MAbs that are specific to H5. The antibodies were obtained by using a purified avian influenza virus type A (H5N1) as an immunogen.

Antibodies specific to influenza A H7 (Cat.# 3HI7)

We provide three MAbs that are specific to H7. The antibodies were raised against purified Influenza A/Netherlands/219/03 H7N7 virus. All of the MAbs detect influenza A H7 hemagglutinin in direct and indirect ELISA.

We investigated the cross-reactivity of the MAbs to other influenza A H subtypes: Influenza A H1N1 (strain A/New Caledonia/20/99), Influenza A H2N2 (strain A/Japan/305/57), Influenza A H3N2 (strain A /Panama/2007/99), as well as to the parainfluenza virus (type 1 Sendai). All of the MAbs demonstrated high specificity to H7 and did not bind to other tested viruses. Cross-reactivity data with the MAb InA334 is provided in Figure 10 and show that the antibody only reacts with H7.

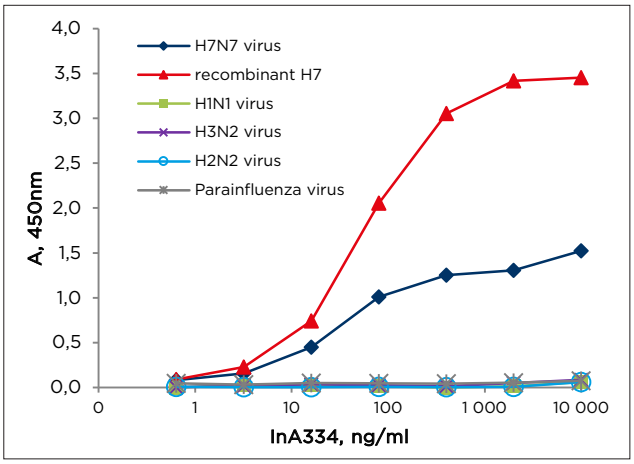

Figure 10. Titration curve of the MAb InA334 that is specific to haemagglutinin H7 in indirect ELISA. Viral antigens 200 ng/well and recombinant H7 (A/Chicken/Netherlands/1/03; a.a.r. 17-527) 10 ng/well.

H7 quantitative sandwich immunoassay. All of the MAbs were tested in sandwich type immunoassays as either capture or detection MAbs. The best pairs of MAbs were selected based on their ability to detect influenza A H7 with high sensitivity using the purified strain of Influenza A/Netherlands/219/03 H7N7 and recombinant H7 as antigens. We recommend using the pairs InA334–InA331 and InA334–InA414. The calibration curves for InA334–InA331 are provided in Figure 11.

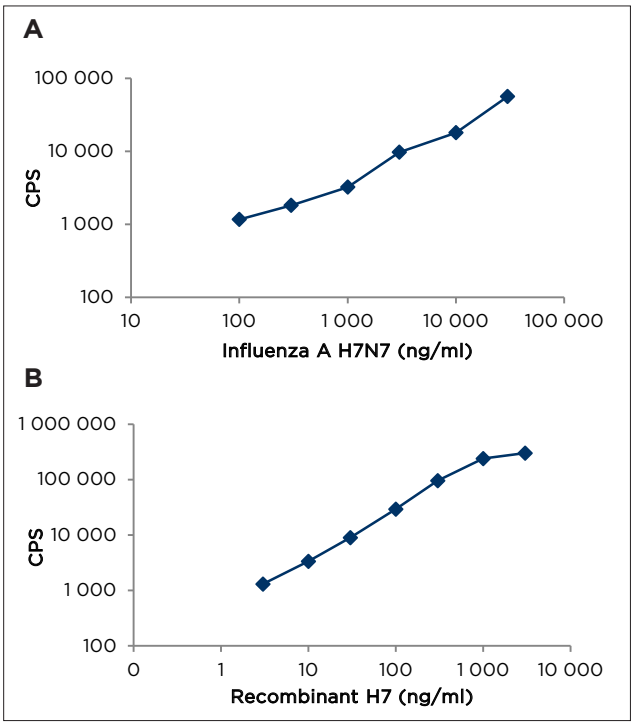

Figure 11. Calibration curves for influenza A haemagglutinin H7 in sandwich fluoroimmunoassays.
Capture MAb: InA334 (1 µg/well)
Detection MAb: Eu^{3+} labelled InA331 (0.1 µg/well)
Incubation time: 45 min
A) Influenza A/Netherlands/219/03 (H7N7)
B) Recombinant H7 (A/Chicken/Netherlands/1/03; a.a.r. 17-527)

Influenza A antigens

We provide nine different influenza A strains (H1 and H3) as antigens. The viruses are purified from the allantoic fluid of 10-12 day old embryonated chicken eggs that are inoculated with different influenza A strains and inactivated with thimerosal and beta propiolactone treatment. The purity of all products is >90 %. These antigens could be used in the detection of antibodies to the influenza A viruses in ELISA, hemagglutination inhibition tests and Western blotting.

The antigens do not cross-react in ELISA with MAbs that are specific to influenza A of other HA subtypes or with MAbs which are specific to influenza B HA or NP. In the hemagglutination inhibition test, the antigens do not cross-react with antisera to different influenza A or B subtypes (Table 1 summarizes the cross-reactivity data for two of the antigens).

Table 1. Hemagglutination test of selected influenza A antigens. H1N1 and H3N2 antigens are only detected with antiserum raised against the corresponding subtype and not with other antisera.

		Rabbit antisera to				
		A/New Caledonia/20/99 (H1N1)	A/St.Petersburg/186/00 (H3N2)	A/Singapore/1/57 (H2N2)	A/swine/1976/31 (HswN1)	B/Tokio/53/99
Virus	A/New Caledonia/20/99 (H1N1)	640	<10	<10	<10	<10
	A/Panama/2007/99 (H3N2)	<10	320	<10	<10	<10

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Influenza A nucleoprotein	3IN5	F8	IgG2a	EIA, IHC
		InA108	IgG1	EIA, WB
		InA180	IgG3	EIA
		InA224	IgG1	EIA
		InA245	IgG2b	EIA, WB
Influenza A haemagglutinin	3IH4	C102	IgG1	EIA, IF, HIT, IHC, haemagglutinin H1
Influenza A haemagglutinin H1	3AH1	InA4	IgG1	EIA, WB
		InA97	IgG1	EIA, WB
		InA134	IgG1	EIA, WB
		InA139	IgG1	EIA, WB
Influenza A haemagglutinin H3	3HG3	InA227	IgG1	EIA, WB
		InA246	IgG2a	EIA, WB
Influenza A haemagglutinin H5	3H5N	15A6	IgG2a	EIA, HIT, Dot blot
		18D5	IgG2a	EIA, HIT, Dot blot
		19C11	IgG2a	EIA, HIT, Dot blot
		8D2	IgG2a	EIA, HIT, Dot blot
		6C8	IgG1	EIA, HIT
		1C7	IgG2a	EIA, HIT
		1B4	IgG2a	EIA
Influenza A haemagglutinin H7	3HI7	InA331	IgG1	EIA
		InA334	IgG1	EIA
		InA414	IgG2b	EIA

ANTIGENS

Product name	Cat. #	Purity	Source
Influenza A (H1N1) virus	8IN73	>90%	A/Taiwan/1/86
Influenza A (H1N1) virus-2	8IN73-2	>90%	A/Beijing/262/95
Influenza A (H1N1) virus-3	8IN73-3	>90%	A/New Caledonia/20/99
Influenza A (H1N1) virus-4	8IN73-4	>90%	A/Solomon Islands/03/06
Influenza A (H3N2) virus	8IN74	>90%	A/Shangdong/9/93
Influenza A (H3N2) virus-1	8IN74-1	>90%	A/Panama/2007/99
Influenza A (H3N2) virus-2	8IN74-2	>90%	A/Kiev/301/94
Influenza A (H3N2) virus-3	8IN74-3	>90%	A/Wisconsin/67/05
Influenza A (H3N2) virus-4	8IN74-4	>90%	A/Brisbane/10/07

Influenza B monoclonal antibodies

HyTest offers a panel of monoclonal antibodies that are specific to influenza B NP, HA and matrix protein 1 (M1).These MAbs work with high affinity and specificity in different immunoassays including direct or indirect ELISA, sandwich immunodetection systems, as well as in Western blotting.

Antibodies specific to influenza B NP (Cat.# 3IF18 and RIF17)

We provide several MAbs that are specific to influenza B NP. The MAb R2/3 (Cat.# RIF17) is produced *in vitro*. The antibodies were raised against the purified influenza virus type B. They are highly specific to influenza B nucleoprotein and they do not cross-react with influenza A NP or other viral proteins that were tested. The low detection limit of our MAbs enables the detection of the virus even in samples with a low influenza B titer. Furthermore, the MAbs can be utilized in rapid influenza B assays. Table 2 shows the epitope specificities of the MAbs and antibody pair recommendations are provided in Table 3.

Table 2. Epitope specificities of MAbs that are specific to influenza B nucleoprotein.

Epitope	MAbs
Fragment 1: (1-80 a.a.r.)	InB12, InB36
Fragment 2: (120-200 a.a.r.)	InB27, InB64
Fragment 3: (240-320 a.a.r.)	InB204, InB210, 2/3
Fragment 4: (480-560a.a.r)	InB114, InB213

Table 3. Antibody pair recommendations for influenza B nucleoprotein sandwich immunoassays.

Capture	Detection
InB12	InB27
InB12	InB64
InB36	InB64

All anti-NP MAbs detect different strains of influenza B in direct and indirect ELISA. The titration curves of the MAb InB114 are shown in Figure 12.

Influenza B quantitative sandwich immunoassay. The MAbs were tested in sandwich type immunoassays as capture and detection MAbs. Recommended pairs (see Table 3) were selected based on their ability to detect several influenza B strains and recombinant influenza B NP with equal specificity and high sensitivity. The strains that were tested were Influenza B/Leningrad/86/93, Influenza B/Tokyo/53/99 and Influenza B/Victoria/504/00. The calibration curve for the pair InB36–InB64 is shown in Figure 13.

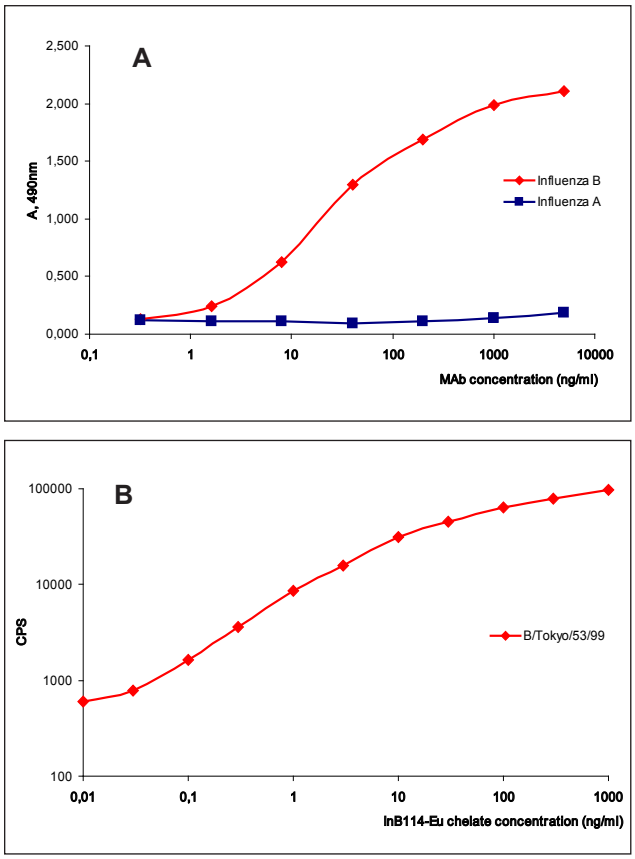

Figure 12. Titration curves of the MAb InB114 that is specific to influenza B NP in indirect (A) and direct (B) ELISA.
Influenza B: Influenza B/Tokyo/53/99 (A: 0.5 µg/well, B: 0.2 µg/well).
Influenza A: Mixture of strains A/Shangdong/9/93 and A/New Caledonia/20/99 (0.5 µg/well).

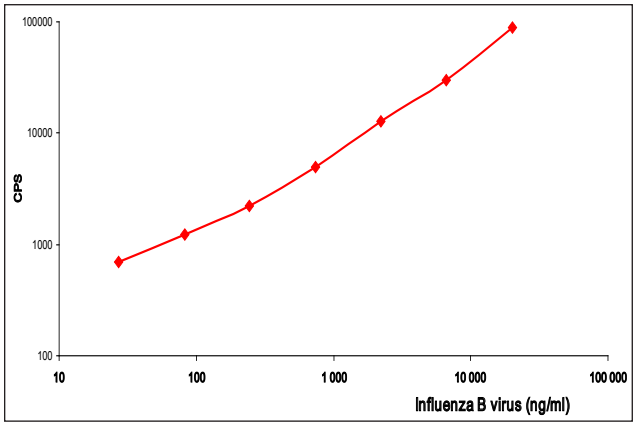

Figure 13. Calibration curve for influenza B NP in a sandwich fluoroimmunoassay.
Capture MAb: InB36 (1 µg/well)
Detection MAb: Eu^{3+} labelled InB64 (0.2 µg/well)
Incubation time: 45 min
Antigen: Influenza B/Tokio/53/99.

MAb R2/3. This MAb (Cat.# RIF17) is produced *in vitro*. The antibody detects the nucleoprotein of the virus and it has been tested with several influenza B strains (see Figure 14). No cross-reactivity was detected when it was tested with nine influenza A strains, three parainfluenza strains, adenovirus (type 6) or respiratory syncytial virus.

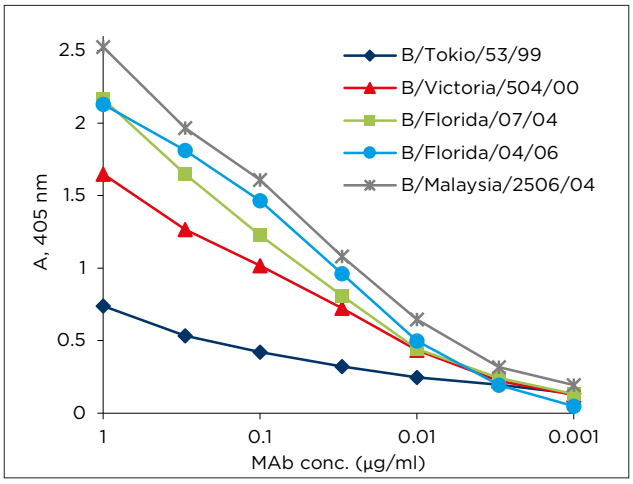

Figure 14. A direct ELISA analysis of the anti-influenza B group antigen (Cat.# RIF17) with different influenza B strains. Antigens were absorbed to the plate in 5 µg/ml concentration and antibody binding was tested using a dilution series of the antibody from 1 µg/ml to 1 ng/ml concentration.

Influenza B NP immunodetection in Western blotting. MAbs detect NP of influenza B in Western blotting after SDS-PAGE in reducing and non-reducing conditions. Western blotting using MAbs InB27 and InB63 is shown in Figure 15.

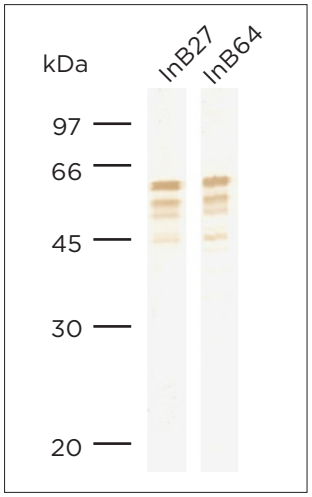

Figure 15. Immunodetection of influenza B NP in Western blotting after PAGE in reducing conditions.
Antigen: Influenza B/Tokio/53/99, 1 µg/well. Antibodies InB27 and InB64, 5 µg/ml. Anti-mouse IgG conjugated with HRP was used as the secondary antibody.

Antibodies specific to influenza B HA (Cat.# 3GH9)

We provide two MAbs that are specific to hemagglutinin HA2. Both MAbs equally detect the hemagglutinin of different influenza B strains in direct and indirect ELISA. The titration curves of the MAb InB190 are shown in Figure 16.

HA immunodetection in Western blotting. All of the MAbs detect the hemagglutinin HA2 chain of different influenza B strains in Western blotting after SDS-PAGE in reducing conditions. Western blotting using the MAb InB190 is shown in Figure 17.

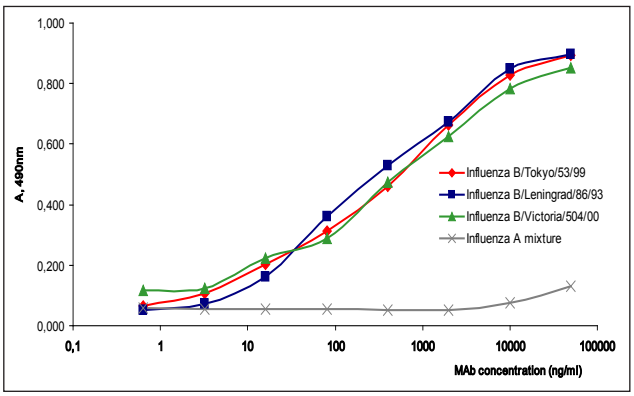

Figure 16. Titration curves of the MAb InB190 that is specific to influenza B HA in indirect ELISA. Antigens: 0.5 µg/well. Influenza A was a mixture of strains A/Shangdong/9/93 and A/New Caledonia/20/99.

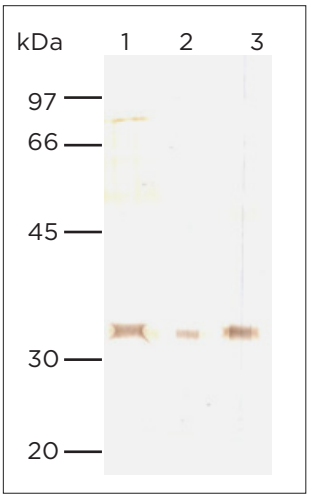

Figure 17. Immunodetection of influenza B HA2 using the MAb InB190 in Western blotting.
Lane 1: Influenza B/Tokio/53/99, 1 µg/well, lane 2: Influenza B/Leningrad/86/93, 1 µg/well, lane 3: Influenza B/Victoria/504/00, 1 µg/well, Primary antibody: MAb InB190 (3 µg/ml), Secondary antibody: Anti-mouse IgG conjugated with HRP.

Antibodies specific to influenza B M1 (Cat.# 3BM17)

We provide two MAbs that are specific to influenza B matrix protein M1. The MAbs detect M1 protein in direct and indirect ELISA. The titration curve of the MAb InB4 is shown in Figure 18.

M1 immunodetection in Western blotting. Both MAbs detect M1 in Western blotting after SDS-PAGE in reducing conditions.

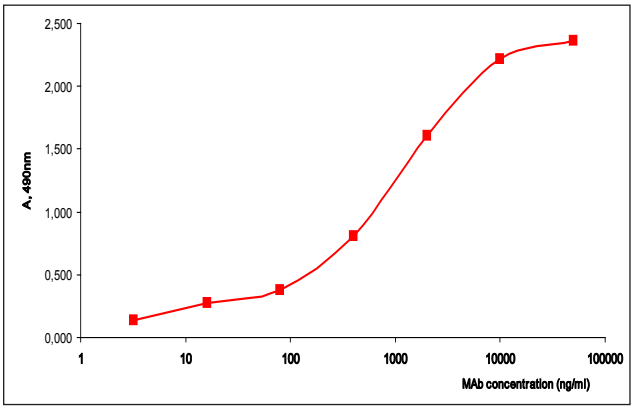

Figure 18. Titration curve of the MAb InB4 that is specific to influenza B M1 in indirect ELISA. Antigen: Influenza B/Tokyo/53/99, 0.5 µg/well.

Influenza B antigens

We provide five different influenza B strains as antigens. The viruses are purified from the allantoic fluid of 10-12 day old embryonated chicken eggs that are inoculated with influenza B strains and inactivated with thimerosal and beta propiolactone treatment. The purity of all products is >90 %. These antigens could be used in the detection of antibodies to influenza B viruses in ELISA, hemagglutination inhibition tests and Western blotting.

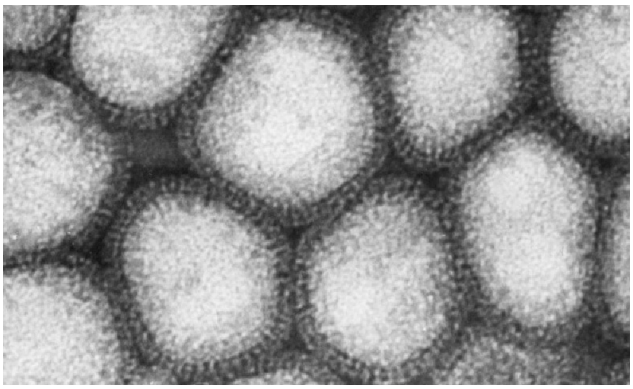

Figure 19. Electron microscopic image of the influenza B virus. (The diameter of the virus particles is 100-120 nm. Magnification 110,000x.

Influenza B antigens do not cross-react in ELISA with MAbs that are specific to influenza A hemagglutinin subtypes (H1N1 or H3N2). In the hemagglutination inhibition test, the antigens do not cross-react with antisera to influenza A H1N1 or H3N2 viruses (Table 4 summarizes the cross-reactivity data for two antigens)

Table 4. Hemagglutination test of selected influenza B antigens. The antigens are only detected with antiserum raised against the corresponding virus and not with other antisera.

		Rabbit antisera to				
		B/Tokio/53/99	B/Victoria/504/00	A/New Caledonia/20/99	A/St.Petersburg/186/00	A/swine/1976/31
Virus	B/Tokio/53/99	320	<10	<10	<10	<10
	B/Victoria/504/00	<10	320	<10	<10	<10

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Influenza B haemagglutinin	3BH9	InB18	IgG2a	EIA, WB, haemagglutinin 2 (HA2)
		InB190	IgG2b	EIA, WB, haemagglutinin 2 (HA2)
Influenza B matrix protein M1	3BM17	InB4	IgG1	EIA, WB
		InB15	IgG1	EIA, WB
Influenza B group antigen	3IF18	2/3	IgG2a	EIA, WB, IF, nucleoprotein
		InB12	IgG2b	EIA, WB, nucleoprotein
		InB27	IgG1	EIA, WB, nucleoprotein
		InB36	IgG1	EIA, WB, nucleoprotein
		InB64	IgG1	EIA, WB, nucleoprotein
		InB114	IgG1	EIA, WB, nucleoprotein
		InB204	IgG1	EIA, WB, nucleoprotein
		InB210	IgG1	EIA, WB, nucleoprotein
		InB213	IgG1	EIA, WB, nucleoprotein
Influenza B group antigen, *in vitro*	RIF17	R2/3	IgG2a	EIA, WB, nucleoprotein

ANTIGENS

Product name	Cat. #	Purity	Source
Influenza B virus-2	8IN75-2	>90%	B/Tokio/53/99
Influenza B virus-3	8IN75-3	>90%	B/Victoria/504/00
Influenza B virus-4	8IN75-4	>90%	B/Malaysia/2506/04
Influenza B virus-5	8IN75-5	>90%	B/Florida/07/04
Influenza B virus-6	8IN75-6	>90%	B/Florida/04/06

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