

HyTest NEWS



Avian Influenza



General

Avian influenza viruses occurring naturally among birds cause avian influenza infection. Usually “avian influenza virus” refers to influenza A viruses found mainly in birds, but infections with these viruses can occur also in humans. Avian influenza was first identified over 100 years ago during an outbreak in Italy. Since then, the disease has cropped up at irregular intervals in all world regions.

There are many different subtypes of type A influenza viruses and they differ because of changes in certain proteins on the surface of the influenza A virus (hemagglutinin [HA] and neuraminidase [NA] proteins). Many different combinations of HA and NA proteins are possible and each combination represents a different subtype. Of the 16 different hemagglutinin types only strains within the H5 and H7 subtypes cause highly pathogenic avian influenza, which is highly 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 %.

Highly pathogenic for mice avian influenza H5N1 strain was prepared as allantoic fluid of SPF chicken embryos. The virus was purified by ultracentrifugation. Inactivated virus vaccine was prepared by ether treatment and homogenization with complete Freund's Adjuvant. Balb/c mice were vaccinated by injection of 10 µl of the vaccine into each hind foot pat. The vaccination was repeated twice within one week. Spleen and lymph nodes were used for hybridoma production. Splenocytes and lymphocytes were fused with Sp2/0 mouse myeloma by PEG-6000 and clones were grown in 96-well plates with selective HAT medium for 10 days. Screening of the MAb producing clones was performed by Hemagglutinin Inhibition (HI) test and by indirect ELISA. Positive clones were recloned using mouse peritoneal macrophages as a feeder. Selected 5 MAbs show high specificity for the hemagglutinin H5 in hemagglutinin inhibition (HI) test. All MAbs are suitable for indirect ELISA. For sandwich immunoassay we can recommend MAbs 19C11 or 11A9 for coating and MAbs 15A6 or 8D2 for detection. MAbs are working also in Dot blot application.

Ordering information:

MAb	Cat.#	Specificity	Subclass	Remarks
8D2	3H5N	Hemagglutinin H5	IgG2a	HIT, EIA, Dot blot
11A9	3H5N	Hemagglutinin H5	IgG2a	HIT, EIA, Dot blot
15A6	3H5N	Hemagglutinin H5	IgG2a	HIT, EIA, Dot blot
18D5	3H5N	Hemagglutinin H5	IgG2a	HIT, EIA, Dot blot
19C11	3H5N	Hemagglutinin H5	IgG2a	HIT, EIA, Dot blot



Anti-avian influenza virus type A monoclonal antibodies:

Host Animal: Mice Balb/c
 Cell line used for fusion: Sp 2/0
 Immunogen: Purified avian influenza virus type A (H5N1)
 Isotype: IgG2a for all MAbs
 Purification: Protein G affinity chromatography
 Specificity: MAbs react with Hemagglutinin H5 in Hemagglutinin Inhibition (HI) test
 Applications: Hemagglutinin Inhibition (HI) test, Indirect ELISA, Dot blot

Table 1. Activity of MAbs in HI test with H5 of different strains.

Samples	H5N1 embryo (RIAH)	H5N1 cell cultural supernatant (RIAH)	H5N2 (Venice)	H5N9 (USA)
MAb 8D2	1:512	>1:4096	1:4096	>1:4096
MAb 11A9	1:128	1:1024	1:1024	>1:4096
MAb 15A6	1:512	>1:4096	1:4096	>1:4096
MAb 18D5	1:1024	>1:4096	1:256	>1:4096
MAb 19C11	1:128	1:1024	1:512	1:1024
Serum ⁺ (RIAH)	1:512	1:2048	1:512	1:1024
Serum ⁺ (USA)	1:32	1:64	1:32	1:2048

Table 2. Cross reactivity of MAbs in HI test with different hemagglutinins (Negative- <1:2).

Antigen	Serum ⁺	Serum ⁻	MAb 8D2	MAb 11A9	MAb 15A6	MAb 18D5	MAb 19C11
H1	1:64	Negative	Negative	Negative	Negative	Negative	Negative
H2	1:256	Negative	Negative	Negative	Negative	Negative	Negative
H3	1:512	Negative	Negative	Negative	Negative	Negative	Negative
H4	1:1024	Negative	Negative	Negative	Negative	Negative	Negative
H6	1:128	Negative	Negative	Negative	Negative	Negative	Negative
H7	1:8	Negative	Negative	Negative	Negative	Negative	Negative
H8	1:512	Negative	Negative	Negative	Negative	Negative	Negative
H9	1:1024	Negative	Negative	Negative	Negative	Negative	Negative
H10	1:4096	Negative	Negative	Negative	Negative	Negative	Negative
H11	1:1024	Negative	Negative	Negative	Negative	Negative	Negative
H12	1:512	Negative	Negative	Negative	Negative	Negative	Negative
H13	1:2048	Negative	Negative	Negative	Negative	Negative	Negative
H14	1:256	Negative	Negative	Negative	Negative	Negative	Negative
H15	1:256	Negative	Negative	Negative	Negative	Negative	Negative