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### H5N1亚型禽流感病毒血凝素基因的克隆与表达

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### Cloning and expression of hemagglutinin gene of H5N1 subtype avian influenza virus

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**摘要** 根据已知H5N1亚型禽流感病毒血凝素(HA)基因序列设计、合成克隆引物.自灭活的云南地方H5N1亚型病毒阳性临床组织样品中提取总RNA,反转录后采用高可信度DNA聚合酶(Pyobest™ DNA Polymerase)扩增HA基因,采用Invitrogen定向表达系统(Champion™ pET directional TOPO expression system)进行克隆表达,纯化获得N末端携带多聚组氨酸标签的重组HA,分子质量约78ku.采用阳性血清经免疫印迹及ELISA分析重组HA的免疫反应性,结果表明重组HA能与H5N1亚型病毒抗血清发生特异性结合,具有良好的免疫反应性.

**关键词:** 禽流感病毒 H5N1亚型 血凝素 表达

**Abstract:** A pair of clone primers was designed and synthesized based on hemagglutinin(HA) gene sequences of known H5N1 subtype avian influenza viruses.HA gene were amplified from total RNA,which had been extracted from inactivated Yunnan H5N1 subtype virus positive clinical tissue sample,by reversing transcriptase-polymerase chain reaction using high proofreading polymerase(Pyobest™ DNA Polymerase),and was expressed using Invitrogen Champion™ pET directional TOPO expression system.Recombinant HA containing polyhistidine(6xHis) tag in N-terminal is about 78 ku in size,which had been obtained and purified.Its immunoreactivity was analyzed by western blot and ELISA using positive serum.The results showed recombinant HA can bind to antiserum against H5N1 subtype virus with specificity and possesses good immunoreactivity.

**Key words:** avian influenza virus H5N1 subtype hemagglutinin expression

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