

제 목	Cloning of Xanthine Oxidase Gene from Mouse Liver cDNA Library
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내 용	<p>Bovine milk xanthine oxidase (E.C.1.1.3.22, XO) purchased from Sigma Chemical Co. had the three protein fragments below 150 kDa on 7.5% SDS-PAGE, which did not show enzyme activity. To remove these fragments, the enzyme preparation was further purified through Sephadex G-200 column chromatography. Two peaks exhibiting enzymatic activity were separated very closely to the void volume, which were revealed as two different enzyme forms, dimeric and monomeric, confirmed by activity staining on native PAGE. Antisera against each of the two enzyme forms were raised by subcutaneous injection at multiple sites on the back of rabbits during 4 weeks. On the immunodiffusion test, it was found that both of the antisera of the two forms could react with each other, which implied that their epitopes were identical. In the Western blot analysis of mouse liver cytosol fraction, it was found that rabbit anti-XO antibody bound well with the protein band of monomeric mouse liver XO of about 150 kDa. Based on this result, mouse liver cDNA library was screened by <i>in situ</i> hybridization with rabbit anti-XO antibody as probe. Through the immunological screening, recombinant phages giving positive signal by the production of XO were selected and further purified. To validate these clones, purified phages were lysogenized in <i>E.coli</i> Y1089 and their lysates were analysed for enzyme activity and immunoreactivity. It was verified that lysates of the purified recombinant phage lysogens exhibited the enzymatic activity as well as bound with XO antibody, when induced by IPTG. The above results assert that selected recombinant phage carries mouse liver XO gene.</p>