

Monoclonal antibody for canine trypsin-like immunoreactivity for development of an immunochromatographic test kit

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Purpose: Present study performed to prepare monoclonal antibodies against canine trypsin-like immunoreactivity (cTLI) for development of an immunochromatographic test kit.

Materials and Methods: The anionic trypsin, cationic trypsin, and chymotrypsin from canine pancreas were purified by using ammonium sulfate fractionation and benzamidine-affinity chromatography, and the levels of their purification were determined by means of sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by silver staining.

Results: The purification fold for anionic trypsin was 108 times when compared with that of the homogenation of pancreas. The molecular weights by SDS-PAGE analysis were approximately 23 kDa for chymotrypsin and approximately 20 kDa for cationic trypsin and anionic trypsin, respectively. Using the purified trypsin-like proteins, ten hybridomas which secret canine trypsin-specific monoclonal antibody were prepared. Klotz plot indicated that 5G2H10G4 and 2F4A11, hybridoma, have high affinity constant (K_a) of 4.1×10^9 and 1.8×10^9 , respectively. Especially, 5F9H3 showed the cationic trypsin-specific binding pattern and its K_a was determined to 4.5×10^9 .

Conclusion: The development of rapid immunochromatographic test kit using these monoclonal antibodies against cTLI will be very useful in the diagnosis of canine exocrine pancreatic insufficiency or canine pancreatitis.

Key words: dog, trypsin-like immunoreactivity (cTLI), monoclonal antibodies, anionic trypsin, cationic trypsin, pancreatitis, exocrine pancreatic insufficiency

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