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Technical Approach to Generate Polyclonal Antibodies Against Bacterially Expressed GST-PYK- C: Evaluation of Cross-Reaction and Recognition in Some Mammalian and Yeast Cell Lysates

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Keywords
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Abstract: Pyk-2 is a protein tyrosine kinase, highly expressed in human hippocampus, dentate gyrus and olfactory bulb. In this study, polyclonal antibodies against the C-terminal of pyk-2, starting from Pro-759, were generated by injecting rabbits with the bacterially expressed GST fusion construct, generated by cloning the C-terminal domain of pyk-2 into pGEX-3X vector and affinity purification to increase the specificity of recognition. Anti-pyk-C was shown to recognize a 123 kD band in Western Blots of 293 cells transfected with pyk-2, as well as PC-12, COS and 3T3 cells and not to cross-react with FAK transfected Sf-21 lysates, in spite of high sequence homology with FAK. This is an early study to develop quantitative immunochemical diagnostic assays, to determine the importance of pyk-2 in human physiology through clinical laboratory investigations. The use of bacterial expression systems in preference to other choices for antigen generation against native mammalian proteins are discussed, in context of the description of 'functional epitope'.

Key Words: pyk-2, Central Nervous System, Molecular Cloning, Gene Fusion, Bacterial Proteins, Antibody Formation, Antibody Specificity, Immunochemistry, Immunoblot-ting.

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