Monoclonal antibodies (MAbs) are important tools to develop methods for bacterial detection. Our goal was to obtain MAbs that react specifically with *Listeria* species. Two female BALB/c mice were immunized initially with approx $10^8$ heat-killed *L. monocytogenes* cells suspended in complete Freund’s Adjuvant (FA). Six consecutive injections of live bacteria ($10^5$ cells) in incomplete FA were performed at 7 days intervals. Splenocytes from mouse with the highest serum conversion were fused with myeloma cell line SP2/0. Hybridoma clone secreting a MAb-3F8 (IgM isotype) was selected and characterized by ELISA and Western blot using a panel of several *Listeria* spp. and other non-*Listeria* bacteria such as *Salmonella*, *Bacillus*, *Escherichia coli*, *Lactobacillus paracasei*, etc. MAb-3F8 reacted with *Listeria* spp. including all 13 *L. monocytogenes* serotypes without reaction with any other bacteria tested. In Western blot, MAb-3F8 reacted with a 30 kDa protein. MALDI-TOF analysis revealed this protein to be fructose 1,6-bisphosphate aldolase (FBA). In fiber optic biosensor, a combination of MAb-3F8 as capture molecule and Cy5 conjugated anti-InlA MAb-2D12 as reporter was highly selective for *L. monocytogenes*. The results suggest that MAb-3F8 has great potential for use in the development of immunochemical methods for detection of *Listeria* spp. including *L. monocytogenes*, and as an analytical tool to study FBA protein in *Listeria*. 