

¹⁰潘秀华,孟宪荣,栗绍文,张超,谢世琦,闫少侠,蔡旭旺.单增李斯特菌胶体金免疫层析试纸条的研制[J].中国食品卫生杂志,2014,26(2):115-119.

单增李斯特菌胶体金免疫层析试纸条的研制

Development of colloidal gold strip for detection of *Listeria monocytogenes*

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中文摘要:

以单增李斯特菌(LM)内化素A蛋白(InLA)单克隆抗体为基础,研制其胶体金免疫层析检测试纸条。方法 采用DNAStar软件对LM inLA全长基因编码蛋白进行抗原表位分析,截取部分inLA基因片段构建原核表达质粒,诱导表达和纯化获得重组蛋白。以该蛋白免疫BALB/c小鼠,筛选高效分泌抗InLA单克隆抗体的杂交瘤细胞株,制备单克隆抗体;以双抗体夹心的原理研制胶体金免疫层析检测试纸条,并对其特异性、敏感性、稳定性进行评价。结果 筛选到2株高效分泌抗InLA单克隆抗体的杂交瘤细胞株,抗体属于IgG1亚类,小鼠腹水抗体效价为1: 64000;研制的试纸条可与

LM发生阳性反应而与非LM李斯特菌、链球菌、鼠伤寒沙门菌、大肠杆菌O157 : H7等食源性致病菌均不发生阳性反应；LM纯培养物敏感性为 2.4×10^5 cfu/ml,模拟猪肉样品敏感性为 4.0×10^6 cfu/ml；4℃保存期可达16周以上。结论 研制的胶体金免疫层析试纸条具有快速、特异、敏感等优点,可以用于样品中LM的快速检测。

Abstract:

To develop a colloidal gold strip for detection of Listeria monocytogenes (LM) based on the monoclonal antibody against LM internalin A (InLA) protein. Methods After analyzing the antigenic epitopes of LM inLA full-length gene encoded protein using DNASTar software, the target inLA gene fragment was selected to construct the prokaryotic expression plasmid, and the recombinant InLA protein was prepared by inducible expression and used to immunize the BALB/c mice. The specific monoclonal antibody against LM InLA protein was prepared. Based on the principle of double-antibody sandwich method, the colloidal gold strip was developed, and its specificity, sensitivity, and stability were evaluated. Results Two hybridoma cell lines were identified to specifically secret anti-InLA monoclonal antibodies, and the antibody subclasses were IgG1 subtype. The antibody titers of acites were 1 : 64000. The colloidal gold strip showed positive reaction with the LM strains, but showed negative reactions with Listeria other than LM, as well as other food-borne bacteria such as Streptococcus, Salmonella typhimurium and EHEC O157 : H7. The detection limits for LM pure cultures and analog samples were 2.4×10^5 and 4.0×10^6 cfu/ml, respectively. The strip could be stored at 4℃ for more than 16 weeks. Conclusion The colloidal gold strip could be used to detect LM in food sample rapidly, sensitively and accurately.

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