

农产品辐照研究·食品科学

庆大霉素单克隆抗体的研制及ELISA分析方法的建立

金仁耀¹, 吴建祥²

1. 浙江工商大学 食品与生物工程学院, 浙江 杭州 310012;
2. 浙江大学 生物技术研究所, 浙江 杭州 310058

摘要: 用与牛血清蛋白(BSA)交联的庆大霉素人工抗原(GM-BSA)免疫的BALB/C鼠脾细胞与SP2/O鼠骨髓瘤细胞融合,经筛选、克隆,得到1株能稳定分泌抗庆大霉素单抗的杂交瘤细胞株(6H8),经鉴定6H8的抗体类型及亚类均为IgG1,其轻链为κ链。制备单克隆抗体腹水,腹水的间接ELISA效价在 1×10^{-7} 以上。该单克隆抗体与庆大霉素结构类似物均无交叉反应,具有高度特异性。以制备的单抗建立间接竞争ELISA方法,其线性回归方程为 $y=16.122\ln(x)-2.0143(R^2=0.9934)$,抑制中浓度 IC_{50} 为 $25.2\mu\text{g} \cdot \text{L}^{-1}$,最低检测限 IC_{20} 为 $3.9\mu\text{g} \cdot \text{L}^{-1}$ 。竞争ELISA方法检测鲜奶中的庆大霉素平均回收率在92%~110%之间。抗庆大霉素单抗的制备和竞争ELISA方法的建立为庆大霉素快速检测奠定了基础。

关键词: 庆大霉素 单克隆抗体 竞争ELISA

PREPARATION OF MONOCLONAL ANTI BODY AND DEVELOPMENT OF AN INDIRECT COMPETITIVE ELISA METHOD FOR GENTAMICIN

JIN Ren-yao¹, WU Jian-xiang²

1. School of Food Science Biotechnology, Zhejiang Gongshang university, Hangzhou, Zhejiang 310012;
2. Institute of Biotechnology, Zhejiang University, Hangzhou, Zhejiang 310058

Abstract: One hybridoma cell line (6H8) secreting monoclonal antibody (McAb) against gentamicin was produced by fusing mouse myeloma cells (SP2/O) with spleen cells from BALB/C which immunized by the artificial gentamicin antigen conjugated with bovine serum albumin (BSA). Isotype and subclass of the monoclonal antibody secreted from the hybridoma cell line (6H8) was classified as IgG1. The light chain of the McAb was identified to be κ chain. The McAb obtained could specifically react with gentamicin and its titre of ascitic fluid detected by indirect ELISA was up to 1×10^{-7} . The result of specificity analysis indicated that the McAb had no cross-reactivity with analogues of gentamicin. Based on the producted McAb, an indirect competitive ELISA was established, and its linear regression equation was $y=16.122\ln(x)-2.0143(R^2=0.9934)$. Inhibition rate analysis showed that IC_{50} and IC_{20} values were $25.2\mu\text{g} \cdot \text{L}^{-1}$ and $3.9\mu\text{g} \cdot \text{L}^{-1}$ gentamicin in PBS buffer, respectively. The mean recovery of gentamicin spiked in milk was from 91% to 110%. The producted anti-gentamicin McAb and established competitive ELISA method could lay the foundation for rapid detection of gentamicin residue.

Keywords: Gentamicin monoclonal antibody competitive ELISA

收稿日期 2012-03-26 修回日期 2012-08-13 网络版发布日期

DOI:

基金项目:

浙江省科技计划项目(2011C22078)

通讯作者:

作者简介:

作者Email:

参考文献:

- [1] 农业部畜牧兽医局. 动物源食品中兽药最大残留限量[J].中国兽药杂志, 2003, 37(4): 15-20
- [2] Posyniak A, Zmudzki J, Niedzielska J. Sample preparation for residue determination of gentamicin and neomycin by liquid chromatography[J]. Journal of Chromatography A,2001,914(1-2):59-66
- [3] Lecaroz C, Campanero M A, Gamazo C, et al. Determination of gentamicin in different matrices by a new sensitive high-performance liquid chromatography-mass spectrometric method[J]. Journal of Antimicrobial Chemotherapy,2006,58(3):557-563
- [4] 刘承伟,罗志辉,陆慰天,于兰,卢昕,赵书林. HPLC-共振瑞利散射联用测定庆大霉素各组分[J].广西师范大学学报(自然科学版),2010,28(3):37-40
- [5] 齐雪琴. 电化学液相色谱分析庆大霉素的方法[J].福建分析测试,2009,16(20):21-22

扩展功能

本文信息

- ▶ Supporting info
- ▶ PDF(1212KB)
- ▶ [HTML全文]
- ▶ 参考文献[PDF]
- ▶ 参考文献

服务与反馈

- ▶ 把本文推荐给朋友
- ▶ 加入我的书架
- ▶ 加入引用管理器
- ▶ 引用本文
- ▶ Email Alert
- ▶ 文章反馈
- ▶ 浏览反馈信息

本文关键词相关文章

- ▶ 庆大霉素
- ▶ 单克隆抗体
- ▶ 竞争ELISA

本文作者相关文章

- ▶ 金仁耀
- ▶ 吴建祥

PubMed

- ▶ Article by JIN Ren-yao
- ▶ Article by WU Jian-xiang

- [6] 陈 艳,梁赤周,吴 斌,邹明强,薛 强,郭良宏. 庆大霉素快速Dot-ELISA检测方法的建立及初步应用[J].中国畜牧兽医,2010,37(9): 237-240
- [7] 王选年,杨艳艳,邢广旭,李青梅,样继飞. 盐酸克伦特罗单抗快速检测试剂盒的研制[J].中国兽医学报,2004,24(1): 75-78
- [8] 范国英,王建华,王自良,张改平,邓润广,杨继飞. 链霉素残留快速检测阻断ELISA试剂盒的研制及其性能测定[J].中国兽医杂志,2008,44(1): 82-84
- [9] 金仁耀,桂文君,吴建祥,王春梅,朱国念. 毒死蜱单克隆抗体的研制[J].浙江大学学报(农业与生命科学版),2006,32(6): 591-597
- [10] JIN R Y, GUI W J, GUO Y R, et al. Comparison of Monoclonal Antibody-based ELISA for Triazophos between the Indirect and Direct Formats[J]. Food and Agricultural Immunology,2008,19(1): 49-60
- [11] Liang C Z, Jin R Y, Gui W J, et al. Enzyme-Linked Immunosorbent Assay Based on a Monoclonal Antibody for the Detection of the Insecticide Triazophos: Assay Optimization and Application to Environmental Samples[J]. Environmental science & technology,2007,41: 6783-6788
- [12] Zhang G P, Wang X N, Yang J F, et al. Development of an immuno chromatographic lateral flow test strip for detection of beta- adrenergic agonist Clenbuterol residues[J]. Journal of Immunology Methods,2006,312(1-2): 27-33
- [13] 刘宣兵,张改平,侯玉泽,邓瑞广,赵 东,李彬彬,柴书军. 庆大霉素人工抗原的合成与鉴定[J].黑龙江畜牧兽医,2008,(12): 64-65
- [14] 吴建祥,林福呈,李德葆,陈正贤,娄沂春. 稻瘟病菌单克隆抗体的研制及其对稻瘟病菌附着胞形成的影响[J].微生物学报,2000,40(6): 638-645
- [15] 青玲,吴建祥,戚益军,周雪平,李德葆. 蚕豆萎蔫病毒单克隆抗体的研制及检测应用[J].微生物学报,2000,40(2): 166-173
- [16] 陈伯权,吴美英,叶群瑞. 几种部分纯化单克隆抗体方法的比较[J].病毒学报,1990,6(2): 122-126
- [17] Wu Jian-xiang, Zhang Shao-en, Zhou Xue-ping. Monoclonal antibody-based ELISA and colloidal gold-based immunochromatographic assay for streptomycin residue detection in milk and swine urine[J]. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology),2010,11(1): 52-60
- [18] Abad A, Moreno M J, Montoya A. Development of monoclonal antibodybased immunoassays to the N-methylcarbamate pesticide Carbofuran[J]. Journal of Agricultural and Food Chemistry,1999,47(6): 2475-2485

本刊中的类似文章

1. 王谦,齐孟文,何方洋,杨根海,曲勃. 抗链霉素单克隆抗体的制备和鉴定[J]. 核农学报, 2004,18(02): 158-160+157
2. 杨艳艳, 职爱民, 刘庆堂, 李靛, 李青梅, 柴书军, 胡晓飞, 邓瑞广, 张改平. 特布他林杂交瘤细胞株的建立及其单克隆抗体制备与鉴定[J]. 核农学报, 2012,(2): 335-339
3. 汪俊强,陈子元,陈传群,吴美文. 单克隆抗体孕酮放射免疫分析的研究[J]. 核农学报, 1989,3(03): 187-192
4. 邹明, 陈杖榴. 抗二氟沙星单克隆抗体可变区基因的克隆及序列分析[J]. 核农学报, 2011,25(6): 1221-1224,1243
5. 宋春美, 李青梅, 刘庆堂, 职爱民, 张改平, 王选年. 喹乙醇单克隆抗体的制备及其免疫学特性的鉴定[J]. 核农学报, 2010,24(4): 777-783
6. 王耀, 胡晓飞, 裴亚峰, 赵东, 刘运超, 张改平, 李志西, 邓瑞广. 赭曲霉毒素A模拟表位的筛选及免疫学鉴定[J]. 核农学报, 2012,(9): 1271-1277
7. 职爱民1 李青梅1 刘庆堂1 柴书军1 赵丽娜1,2 胡晓飞1 杨艳艳1. 西马特罗杂交瘤细胞株的建立及其单克隆抗体制备和鉴定[J]. 核农学报, 2010,24(5): 1011-1014
8. 吴雅欣, 许园园, 潘家荣. 毒死蜱单克隆抗体的制备及鉴定[J]. 核农学报, 2009,23(2): 341-344
9. 赵银丽;王建华;王自良;滕蔓;刘庆堂;邓瑞广;范国英;张改平;. 抗恩诺沙星单克隆抗体杂交瘤细胞株的筛选及竞争ELISA试剂盒的研制[J]. 核农学报, 2008,22(06): 898-903
10. 张海棠;王自良;邓瑞广;钟华;范国英;. 莱克多巴胺单克隆抗体的研制及阻断ELISA检测方法的建立[J]. 核农学报, 2008,22(06): 904-908
11. 刘宣兵;鲁晓翠;张改平;杨小保;侯玉泽;邓瑞广;刘庆堂;职爱民;宋春美;. 磺胺二甲氧嘧啶单克隆抗体的制备及其免疫学特性鉴定[J]. 核农学报, 2008,22(05): 739-744
12. 范国英;王建华;钟华;杨艳艳;邓润广;张改平;. 抗链霉素单克隆抗体杂交瘤细胞株的建立及其免疫学特性鉴定[J]. 核农学报, 2007,21(05): 506-509
13. 王文珺;许艇;高宏斌;赵继勋;张国中;李季;. 人工雌性激素己烯雌酚单克隆抗体的制备及表征[J]. 核农学报, 2007,21(01): 79-82
14. 王自良;张改平;杨艳艳;张海棠;王选年;邓瑞广;. 抗苯巴比妥单克隆抗体杂交瘤细胞株的建立及其免疫学特性鉴定[J]. 核农学报, 2006,20(04): 336-340+317
15. 曲勃,齐孟文. 莱克多巴胺单克隆抗体的制备与初步鉴定[J]. 核农学报, 2005,19(05): 393-396