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论文

硫代磷酸二乙酯类农药半抗原设计及抗体识别特性

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摘要:

通过分析硫代磷酸二乙酯类农药的结构特点, 设计并合成了系列半抗原; 采用活泼酯法将半抗原分别与牛血清蛋白(BSA)和卵清蛋白(OVA)偶联制备了系列免疫原和包被原; 通过免疫新西兰大白兔获得了相应抗硫代磷酸二乙酯类农药的类特异性抗体. 建立检测硫代磷酸二乙酯类农药的间接竞争酶联免疫分析(ELISA)方法, 分析探讨了免疫半抗原结构对抗体特性的影响, 并阐述了包被半抗原结构对ELISA灵敏度的影响规律. 结果表明, 手臂取代位置在苯环对位且手臂较短的免疫原具有较好的免疫效果, 同时异源包被可以显著提高ELISA方法的灵敏度. 由抗体PAb-H1和包被原H6-OVA建立的间接竞争ELISA方法可以同时检测7个广泛使用的有机磷农药, 其半抑制浓度(IC₅₀)分别为蝇毒磷(0.013 mg/L)、对硫磷(0.348 mg/L)、啶硫磷(0.022 mg/L)、三唑磷(0.035 mg/L)、甲拌磷(0.751 mg/L)、除线磷(0.850 mg/L)及辛硫磷(1.301 mg/L), 最低检测限符合国内外相关有机磷药物最大允许残留限量标准(MRLS)的检测要求.

关键词: 有机磷农药; 类特异性抗体; 异源包被; 酶联免疫分析

Design of Generic Haptens for *O,O*-Diethyl Phosphorothioate Pesticides and Recognition Properties of Antibodies

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Abstract:

Class-specific polyclonal antibodies for *O,O*-diethyl phosphorothioate pesticides had been generated. A series of generic haptens were synthesized and coupled to bovine serum albumin(BSA) and ovalbumin(OVA) for immunogens and coating antigens, respectively. New Zealand rabbits were immunized with immunogens to obtain corresponding polyclonal antibodies. The effects of hapten structure on antibodies properties and hapten heterology on ELISA sensitivity were also investigated. The results show that haptens with simple spacer-arm can generate antibodies with desired properties, and hapten heterology can improve ELISA sensitivity significantly. The polyclonal antibody PAb-H1 shows high sensitivity to seven commonly used *O,O*-diethyl phosphorothioate pesticides in an indirect competitive ELISA using a heterologous coating antigen(H6-OVA). The 50% inhibition value(IC₅₀) is 0.013 mg/L for coumaphos, 0.348 mg/L for parathion, 0.022 mg/L for quinalphos, 0.035 mg/L for triazophos, 0.751 mg/L for phorate, 0.850 mg/L for dichlofenthion and 1.301 mg/L for phoxim, respectively. And the limit of detection(LOD) is lower than MRLS of all the seven *O,O*-diethyl phosphorothioate pesticides in food and agricultural products. Thus, this indirect ELISA with high sensitivity can potentially be used to develop commercial ELISA kits for a sensitive and rapid detection of *O,O*-diethyl phosphorothioate pesticides residues.

Keywords: Organophosphorus pesticide; Generic hapten; Heterology coating; Enzyme-linked immunosorbent assay(ELISA)

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