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人工雌激素己烯雌酚酶联免疫分析方法的建立

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摘要 本实验旨在初步建立人工合成雌激素己烯雌酚(DES)的酶联免疫吸附分析(icELISA)方法。实验首先合成了己烯雌酚的两种半抗原正丁酸己烯雌酚单醚(DES-CP)、乙酸己烯雌酚单醚(DES-CME)。DES-CP与牛血清蛋白(BSA)偶联作为免疫原免疫BALB/c雌性小鼠并进一步制备了DES单克隆抗体,表征了单克隆抗体的亲和力和特异性。在ELISA优化的条件下,方法的抑制中浓度为 IC₅₀=9.8 ng/mL,检测限

IC₂₀=2.3 ng/mL,工作浓度范围为 2-42 ng/mL. 抗体与己烷雌酚、双烯雌酚的交叉反应分别为 44%, 27%, 与天然雌激素雌二醇的交叉反应小于0.1%. 评估了分析缓冲液中盐离子浓度、pH、有机溶剂含量等因素对分析方法的影响。本分析方法应用于水样的添加实验, 回收率符合分析精度要求。HPLC法对实验结果进行了确证,证明了ELISA应用于水样分析的可靠性。

关键词 <u>己烯雌酚,酶联免疫吸附分析,单克隆抗体,半抗原合成,水样</u> 分类号

Development of Monoclonal Antibody-based Enzyme-linked Immunosorbent Assay to the Estrogen Diethylstilbestrol

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Abstract Diethylstilbestrol (DES) is a synthetic estrogen that has ever been used worldwide. Polyclonal antibodies (PAbs) were used in immunoassay for detection of DES residues in environmental and agricultural samples in previous paper. In this paper, an indirect competitive enzyme-linked immunosorbent assay (icELISA) was developed based on monoclonal antibody (MAb) for the determination of diethylstilbestrol. Mono-*o*-carboxypropyldiethylstilbestrol (DES-CP) and mono-*o*-carboxymethyldiethylstilbestrol (DES-CME) were synthesized to be haptens. DES-CP was coupled to bovine serum albumin (BSA) to be an immunogen in BALB/c female mouse for MAb production. The MAb was characterized for specificity and affinity to DES in icELISA. Under the optimum condition, the icELISA showed an IC₅₀ of 9.8 ng/mL, the limit of detection (IC₂₀) of 2.3 ng/mL and a working range of 2—42 ng/mL. Hexestrol and dienestrol exhibited cross-reactivity values were 44% and 27%, respectively. Cross-reactivity of natural estrogen 17β-estradiol was less than 0.1%. The influences of some factors such as salt concentration, pH and organic solvent concentration on the assay were evaluated. The concentrations of DES in the fortified water samples determined by the assay were correlated well with the fortification levels. The results were confirmed with analysis by HPLC.

Key words <u>diethylstilbestrol</u> <u>enzyme-linked immunosorbent assay</u> <u>monoclonal antibody</u> <u>hapten synthesis</u> <u>water</u> <u>sample</u>

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