Produksi antibodi poliklonal anti aflatoksin B1 dan aplikasinya sebagai konjugat sensor untuk deteksi aflatoksin B1 = Production of polyclonal antibody against aflatoxin B1 and its application as conjugate sensor for aflatoxin B1 detection / Wiyogo Prio Wicaksono

Wiyogo Prio Wicaksono, author

Abstrak

[ABSTRAK
Antibodi poliklonal anti aflatoksin B1 telah berhasil diproduksi pada hewan uji kelinci betina New Zealand White setelah diimunisasikan hapten aflatoksin B1-CMO yang dikonjugasikan dengan Bovine Serum Albumin (BSA) sebagai antigen. Hapten aflatoksin B1-CMO disintesis menggunakan metode karbodiimida dengan substrat aflatoksin B1 dan carboxymethyl hydroxylamine hemihydrochloride (CMO) sebagai linkernya. Hasil karakterisasi kromatografi lapis tipis dengan nilai Rf rata-rata sebesar 0.395, spektrum UV-Visibel dengan puncak λ maks pada 362, 264, 218 nm, spektrum IR dengan puncak 3448.126 cm-1 (3000-3600 cm-1) : OH, pada 1632.249 cm-1(1540-1725 cm-1) : C=O, dan 1642.451 cm-1 (1640-1690 cm-1) :C=N (Oksim), dan hasil fragmentasi spektrometri massa (MS/MS) pada m/z 386, 368.2, 310 membuktikan hapten aflatoksin B1-CMO berhasil disintesis. Hapten ini kemudian dikonjugasikan dengan BSA membentuk antigen aflatoksin B1-BSA (AFB1-BSA) sebelum diimunisasikan ke kelinci. Spesifitas antigen AFB1-BSA terhadap antibodinya dan uji konjugasi hapten ke BSA menunjukkan hasil positif menggunakan uji Dot Blot Immunoassay dengan konsentrasi BSA di dalam antigennya sebesar 1.74 mg/mL. Serum darah kelinci berdasarkan uji Agar Gel Precipitation Test (AGPT) positif mengandung antibodi poliklonal anti aflatoksin B1 setelah dua pekan (hari ke-11) sejak imunisasi primer antigen AFB1-BSA dilakukan. Dari serum darah bleeding panen, diperoleh konsentrasi antibodinya sebesar sebesar 2.19 mg/mL. Immunokromatogafi strip tes berhasil dibuat dengan nanopartikel iridium oksida (IrO2 NPs) sebagai kandidat label antibodinya dan dapat digunakan untuk mendeteksi sampel H IgG pada rentang 0.1 μg/mL sampai 10 μg/mL. Studi pendahuluan ini menunjukkan bahwa perangkat strip tes ini dapat digunakan untuk aplikasi konjugat sensor antibodi anti aflatoksin B1-nanopartikel iridium oksida untuk deteksi aflatoksin B1.
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ABSTRACT
Polyclonal antibody against aflatoxin B1 have been successfully produced in New Zealand White Rabbit after immunized by hapten of aflatoxin B1-CMO conjugated with Bovine Serum Albumin (BSA) as antigen. Hapten of aflatoxin B1-CMO was synthesized using carbodiimide method with afltoksin B1 as substrate and carboxymethyl hydroxylamine hemihydrochloride (CMO) as its linker. The characterization results of thin layer chromatography with Rf value of 0.395, the spectrum of UV-Visible with λ max peaks at 362, 264, 218 nm, the IR spectrum with peak at 3448.126 cm-1 (3000-3600 cm-1): OH , 1632.249 cm-1(1540-1725 cm-1): C = O, 1642.451 cm-1 (1640-1690 cm-1): C = N (oxime), and the results of mass spectrometry fragmentation (MS / MS) at m/ z of 386, 368.2, 310 proved that hapten of aflatoxin B1 -CMO successfully synthesized. Then, the hapten was conjugated to BSA to form antigen of aflatoxin B1-BSA (AFB1-BSA) before immunized to rabbits. The specificity of antigen of AFB1-BSA to its antibody and the confirmation

of hapten-BSA conjugated showed positive results using dot blot immunoassay with BSA concentration in the antigen of 1.74 mg/mL. Based on Agar Gel Precipitation Test (AGPT) shown the rabbit blood serum resulted positive for polyclonal antibody against aflatoxin B1 after two weeks (day 11st) since the primary immunization of its antigen. From blood serum bleeding at harvest obtained the concentration of antibodies was 2.19 mg / mL. An Immunochromatogaphic test strip was successfully fabricated using iridium oxide nanoparticles (IrO2 NPs) as a labeled antibody candidate and can be used to detect the IgG H sample between of 0.1 μg/mL to 10 μg/mL. This preliminary study shown that the device can be used for applications of antibody against aflatoxin B1-nanoparticle iridium oxide conjugate for detection of aflatoxin B1

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