Tandem bioluminescent enzyme immunoassay for BDNF and NT-4/5

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We have developed the highly sensitive simultaneous bioluminescent assay of acetate kinase (AK) and pyruvate phosphate dikinase (PPDK) using a firefly luciferase-luciferin reaction, and applied this assay to a tandem bioluminescent enzyme immunoassay (BL-EIA). Recently, Nelson et al. have reported that neonatal blood concentration of VIP, CGRP, BDNF, NT-4/5 were higher in autistic spectrum than in control children1). Therefore, measurements of these four factors in neonatal blood are made possible to diagnose and care for autism in early stage. In this study, we established highly sensitive tandem BL-EIA for BDNF and NT-4/5. In the proposed assay, we added 50 µL of standard or sample solutions to the wells of microtiter plate were coated with anti-BDNF and anti-NT-4/5 antibody, and incubated for overnight at 4 °C. After washing, we added 50 µL of FITC labeled anti-BDNF and biotin labeled anti-NT-4/5 antibody to the plate. After incubation for 3hr at room temperature and then washing the plate, we added 100 µL of AK labeled anti-FITC and PPDK labeled anti-biotin Fab’ antibody to the plate and incubated for 1hr at room temperature. After washing, the plate was assayed by simultaneous bioluminescent detection method2). The measurable ranges of BDNF and NT-4/5 were 4.9 – 40000 and 31.25 – 2000 pg/mL, the detection limits (at blank + 3 SD) of BDNF and NT-4/5 were 1.2 and 11.4 pg/mL, respectively. The intra-assay coefficients of variation of BDNF and NT-4/5 with each standard point were 1.8 – 9.8 % (n=8) and 2.3 – 6.4 % (n=8), respectively.