

Detection of a heavy metal exposure biomarker, metallothionein in Javanese medaka (*Oryzias javanicus*) using scFv-immobilized protein chip

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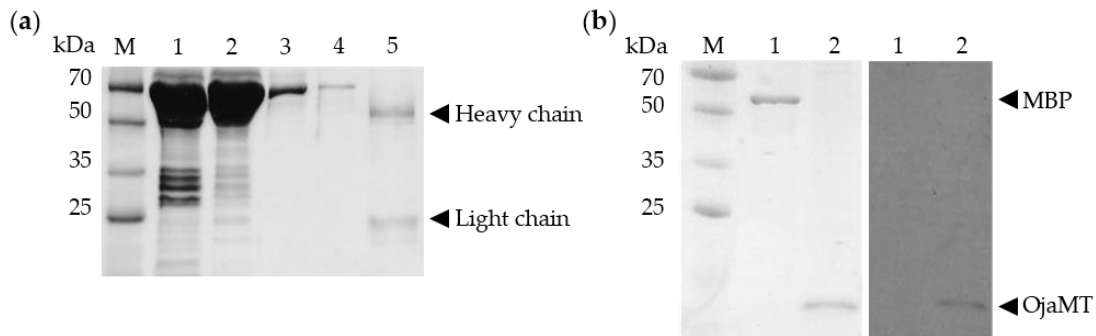


Figure S1. Production and characterization of anti-OjaMT monoclonal antibody (mAb). (a) Purification of anti-OjaMT mAb was analyzed by SDS-PAGE. Lane 1, hybridoma media; lane 2, protein G column flow through; lane 3 and 4, wash fraction; lane 5, elution; M, protein weight marker. (b) SDS-PAGE (left) and western blot analysis using the anti-OjaMT mAb as the primary Ab (right). Lane 1, control protein (maltose binding protein, MBP); lane 2, OjaMT; M, protein weight marker.

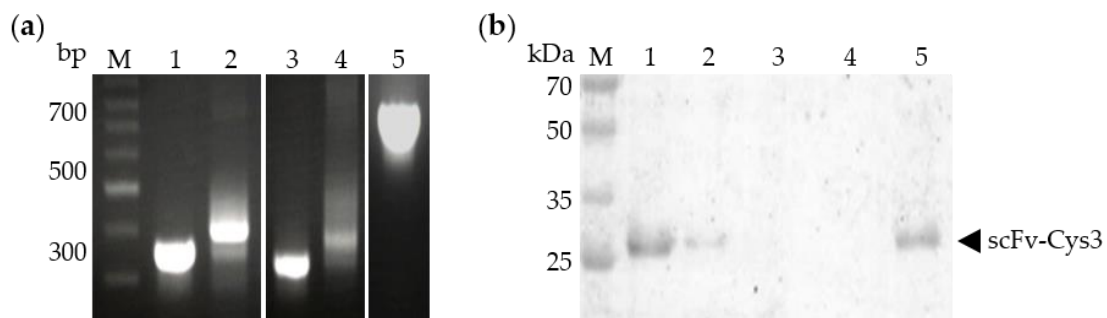


Figure S2. Preparation of the recombinant anti-OjaMT scFv construct. (A) The recombinant anti-OjaMT scFv antibody were analyzed by DNA electrophoresis. Lane 1, heavy chain; lane 2, linker assembled heavy chain; lane 3, light chain; lane 4, linker assembled light chain; lane 5, final assembly of recombinant anti-OjaMT scFv constructs; M, DNA ladder. (C) Purification of anti-OjaMT scFv was analyzed by SDS-PAGE. Lane 1, before refolding sample; lane 2, Ni-column flow through; lane 3 and 4, wash fraction; lane 5, the final refolded recombinant anti-OjaMT scFv-Cys3; M, molecular weight marker.

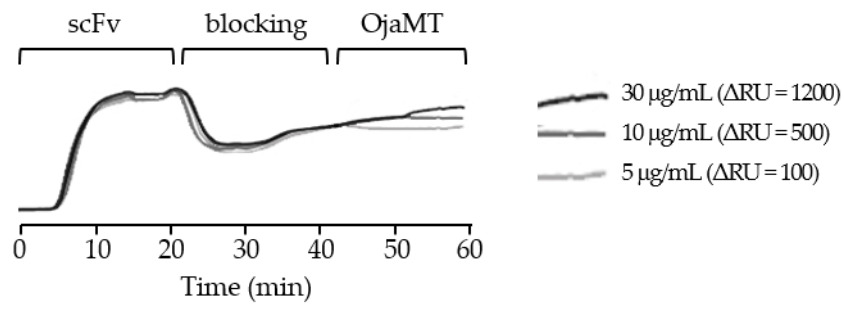


Figure S3. OjaMT detection using SPR chip immobilized with recombinant anti-OjaMT scFv. Sensitivity of scFv-immobilized sensor chip was confirmed through the change of RU value after OjaMT was exposed in the concentration range of 5 µg/mL to 30 µg/mL.