Development of an indirect competitive enzyme-linked immunosorbent assay for formononetin and its application in a cell-based assay using MC3T3-E1 cells

Abstract

Formononetin (FMN) is a methoxy isoflavone found abundantly in leguminous plants and associated foods. Several analytical methods have been developed to detect FMN. However, they are costly, complicated, and time-consuming. This study describes an indirect competitive enzyme-linked immunosorbent assay (icELISA) to determine FMN content in food samples using a monoclonal antibody (mAb) against FMN produced by a newly established hybridoma cell line. Validation studies were conducted, and this assay was found to be sufficiently reliable, with an analytical measurement range of 19.53–1250 ng/mL and a detection limit of 17.42 ng/mL. Furthermore, icELISA was successfully applied for a cell-based assay in which the amount of FMN and ononin uptake was quantified in MC3T3-E1 cells. Hence, icELISA is a simple and reliable method for the detection and quantification of FMN, as well as elucidation of its functions and underlying mechanisms of action.