

## **New strategy using monoclonal antibody on natural product investigation**

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Although the immunoassay system using monoclonal antibody (MAb) is now indispensable for various investigations, it is not so familiar for bioactive compounds having small molecular weight in the field of TCM. Recently rapid, simple, highly sensitive and reproducible assay systems are required for quantitative and/or qualitative analytical techniques in medicinal plant investigations and TCM area in ng and pg scale.

In our ongoing study on the medicinal plant project, we have produced MAbs against forskolin, solamargine, opium alkaloids, marijuana compounds, glycyrrhizin, crocin, ginsenoside Rb1, Rg1 and Re, sennoside A and B, saikosaponin a, paeoniflorin, berberine, artemisinin, aristolochic acid and so on, and applied them for ELISA, immunoaffinity chromatography, eastern blotting, immunocytochemical localization and scFv formation. In this symposium we focus newly established eastern blotting, immunoaffinity concentration (knockout extract) and scFv preparation and its application.

### **Eastern blotting**

Since small molecular compound like saponin can not conjugate to a membrane, no success of staining has been reported. We reached to a new idea that a saponin was divided into two functions, sugar and aglycone moieties. Sugar part can be oxidized to give aldehyde group which is fixed to membrane. Finally we succeeded to stain the aglycone part by MAb. Double staining by two MAbs indicated two coloring, blue and purple which distinguish aglycone differences.

A newly developed eastern blotting can detect saponins by MAb after developing on PES membrane and stained directly. The profile of saikosaponins by the new eastern blotting system will be explained.

### **Immunoaffinity concentration (knockout extract)**

Immunoaffinity column conjugated with MAb which is prepared via hydrazone conjugation with gel can be used for immunoconcentration resulting in high sensitive quantitative analysis of target compound even if low concentration, and makes it

possible to isolate the target compound by one step. The washed out extract was named as knockout extract. We will discuss about how to use the knockout extract.

#### **Analysis by scFv**

The recombinant scFv was expressed in culture medium. The anti-sennoside B (SB) scFv displayed binding activity and cross-reactivity as its parent MAb. The expressed scFv was applied for quantitative ELISA to determine the SB concentration in rhubarb root and cassia leave samples same as that of parent MAb.

From above evidences it became clear that MAb can be used in the field of TCM for qualitative and/or quantitative analysis due to the high specificity, high sensitivity and soft for circumstance because of no need of organic solvent. Moreover, the application of scFv may open a new system for the breeding of medicinal plants in order to increase the antigen molecule concentration because no need of much gene construction for biosynthetic enzymes.