

## Evaluation of RAR Binding Activity Materials in Azolla with Yeast Two Hybrid Assay

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### Extended Abstract

A great variety of chemicals are present in environment. In particular effect to give the human, an animal and an ecosystem with hormone-disrupting chemicals is concerned. For example, the substance called 17 $\beta$ -estradiol is a kind of the female sex hormone called estrogen. The abnormality of the generative organ is found excessively by binding to an estrogen receptor. Also, it has been reported the retinoic acid produces malformation for frame formation by binding to a receptor excessively. Resemblance active substance binding to these receptors, an unknown active agent, unexpected product are present in environment, and there is multiplex exposure. It is difficult to distinguish these by instrumental analysis. Therefore it is an organism reply, the bioassay that can evaluate complex effect comprehensively to be used. Not only we performed environmental monitoring using the yeast two-hybrid method which is one of the bioassay, but also we applied it to the component analysis of the plant.

Yeast Two-Hybrid Assay is bioassay technique to use the recombination yeast which introduced a fusion protein of GAL4DNA binding domain (GAL4 DBD) of ligand binding domain (LBD) of nuclear receptors such as sex hormone or the thyroid hormone (Shiraishi et al., 2000, Kamata et al., 2008). The yeast Two-hybrid Assay method which we used in this study was carried out by the method that improved a traditional approach in our laboratory.

In a previous study, we investigated Azolla [*Azolla cristata*  $\times$  *filiculoides*] of the aquatic fern plant which showed RAR binding activity (Sawabe et al., 2012). Red and green leaves of Azolla were extracted with methanol for one week, respectively. The methanol extract was treated with organic solvents, and the extracts examined RAR binding activity. Remarkable activity was separated over silica gel column chromatography.

### References

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