

On-Bead Screening of a Library to Detect Host-Guest Complexation by an Aniline Reporter.

Miwa Kubo, Ryosuke Nishimoto, Masanori Doi, Mitsuaki Kodama, Hideaki Hioki*

*Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho,
Tokushima 770-8514, Japan,*

Email:hioki@ph.bunri-u.ac.jp

Experimental procedure for the screening of the library **13a for binding to a aniline-labeled tripeptide **16a**.**

5 mg of the solid supported library **13a** (ca. 3 copies for each sequence) was preincubated with 0.6 mL of phosphate buffer (pH 6.86) in an Eppendorf tube for 30 min. The buffer was decanted and 0.6 mL of a guest peptide **16a** ($130 \mu\text{mol L}^{-1}$ in the phosphate buffer) was added to the tube. After agitation for 12 h, the mixture was poured into 0.5 mL of UltrafreeTM MC microcentrifuge tube (0.45 μm filter unit).¹ The resin was filtered and washed several times with the phosphate buffer by using centrifuge until the washing buffer was not active for the Trinder reaction. The resin was transferred to an Eppendorf tube. 0.2 mL of 4-aminoantipyrine (1.0 mmol L^{-1} in water) and 0.2 mL of horseradish peroxidase (10 unit mL^{-1} in water) were added to the tube. After shaking several times, 0.2 mL of H_2O_2 (1.0 mmol L^{-1} in water) was added and incubated for 5 min at 38 °C. The beads were placed onto a Petri dish. The purple colored beads were isolated manually under a low-power microscope. Peptide sequence on colored beads was identified as previously reported manner.²

Note and Reference

1. UltrafreeTM MC microcentrifuge tube was purchased from Millipore Corporation.
2. M. Kubo, E. Nashimoto, T. Tokiyo, Y. Morisaki, M. Kodama, H. Hioki, *Tetrahedron Lett.*, 2006, **47**, 1927-1931.