

# Development of the rapid chromophore solid phase peptide reaction assay (C-SPRA) for sensitization assessment as alternatives to animal testing

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The direct peptide reactivity assay (DPRA) detects binding of test chemical samples to lysine or cysteine of the test peptides as a prominent alternative to animal testing for sensitization. However, DPRA cannot measure binding of poorly water-soluble samples to the test peptides, and the HPLC step in DPRA is time-consuming and technical in comparison with other general measurements. Recently, we had constructed the chromophore solid phase peptide reaction assay (C-SPRA) as an alternative skin sensitization assay system to solve these problems. In this study, we further improved C-SPRA system for detecting various and numerous samples in a short period of time (a few hours).

At first, we selected a microbead for immobilization of the test peptides, PEGA (Poly[acryloyl-bis (aminopropyl) polyethylene glycol]) resin was selected as much more suitable for the quick reaction. Then we optimized the protocol. Especially, the preparation of the peptide-microbeads before the assay was simplified. Using PEGA resin we found that in the preparation we could skip the swelling step every time. These studies effectively modified the conventional C-SPRA. Our method allows a next-generation high-throughput alternative measurement to animal testing for in vitro skin sensitization rapid assessment with easy handling in a few hours.