Title: **Development and validation of the Direct Peptide Reactivity Assay (DPRA) for the measurement of skin sensitization**

Posada a punt i Validació de l'assaig in vitro de sensibilització cutània (DPRA)

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There is a recent concern about not experimenting with animals in skin sensitization, and new *in vitro* methods are being developed to carry it out. One of these methods is DPRA (Direct Peptide Reactivity Assay) which is based on the initial event of skin sensitization produced by the reaction between the allergen and the skin proteins. More specifically, this method studies the covalent union created from the reaction of the electrophilic group present in most of the different types of allergens and the nucleophilic group of each of the two synthetic heptapeptides employed, one containing Cysteine (group thiol as side chain) and the other containing Lysine (primary amine group as side chain). The main objective of this test is to discriminate between a sensitizing substance and a non-sensitizing substance. The way in which it is evaluated is by calculating the mean of the percent peptide depletion for each reagent separately, by means of High Performance Liquid Chromatography (HPLC) with UV detection. This test allows to predict what type of reactivity is expected for each of the reagents classified as sensitizing (high, moderate or low reactivity).

This DPRA method has been successfully implemented and fit-for-purpose validated in the analytical laboratory of a cosmetic manufacturer, using a set of test chemicals that have been correctly classified considering the existing information of the *in vivo* results from the LLNA test (Local Lymph Node Assay), based on the action of these chemicals in the local lymph node of a specific species of mice.

Once the test is validated and as a second part of the project, a battery of reagents used by the company in different products has been analysed. These have been grouped into three sets: preservatives, active ingredients and perfumes.

Keywords: Skin sensitization, DPRA, Cysteine, Lysine, HPLC, UV.