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ANALYSIS OF HUMAN ERYTHROCYTE MEMBRANE AND CYTOSOLIC SUB-PROTEOMES BY 2-DE

G Alvarez-Llamas[1], F de la Cuesta[1], VM Darde[2], MG Barderas[3] and F Vivanco[1,4]

[1]Department of Immunology. Fundacion Jiménez Díaz, Avda. Reyes Catolicos 2, 28040, Madrid, Spain;
[2]Proteomic Unit. Hospital Nacional de Paraplejicos, SESCAM, Finca la Peraleda, 45071, Toledo, Spain;
[3]Department of Vascular Pathophysiology. Hospital Nacional de Paraplejicos, 45071, Toledo, Spain;
[4]Department of Biochemistry and Molecular Biology I, Universidad Complutense, Avda. Complutense s/n, 28040, Madrid, Spain.

Despite of its simple structure, high hemoglobin content present in erythrocytes enormously difficults their proteomic analysis. We investigate here different strategies for isolation of human membrane and cytosolic fractions of red blood cells and their influence on proteome profiling by 2-DE, paying particular attention to hemoglobin removal.

In a first attempt, the use of "2-D Sample Prep for Membrane Proteins" kit was directly applied to the intact erythrocyte cell. However, the high content of hemoglobin greatly interfered with protein detection in both fractions. An hemoglobin depletion approach based on Hemoglobind® reagent, combined with an additional desalting step prior to IEF, was investigated, resulting in the analysis of both membrane and cytosolic sub-proteomes by 2-DE without major interference of hemoglobin. This methodology provided a clear improvement in 2-DE pattern of the membrane and cytosolic proteins compared to gels obtained from hypotonic lysis isolation. However, cross-contamination between the two fractions was clearly seen. The two strategies were then combined: membrane and cytosolic fractions were first obtained by hypotonic lysis; membrane proteins were further solubilized, purified and desalted on commercial mini-colums (no hemoglobin depletion was needed) and cytosolic fraction was submitted to hemoglobin depletion and further desalting.

The possibility of unselectively remove minor proteins together with hemoglobin was also investigated by SDS-PAGE and MALDI-TOF-MS.

In conclusion, we propose a new strategy for 2-DE analysis of human erythrocyte membrane proteins, based on a commercial extraction and purification kit with variations. Besides, a novel approach for hemoglobin depletion is provided in order to analyse the proteome of cytosolic fraction.