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Western blot, ELISA and enzymatic assays of reference proteins for subcellular fractionation

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Protocol Information

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Summary: Subcellular fractionation of mammalian cells has been applied for the study of morphology, composition, structure and interactions between organelles, cellular and molecular biology and, more recently, the cell composition through omics approaches ?. Advantages of fractionation comprise but are not limited to obtaining fractions enriched in certain compartments for the study of cellular processes in vitro ?, locate and track proteins ? and analyze post-translational modifications (PTM) of proteins processed along the secretory pathway ? and protein composition of organelles?. Despite the existence of a wide variety of cell fractionation protocols and techniques, most of them are based on the identification or assignment of a certain isolated fraction to one or more subcellular compartments from the enrichment of this fraction in certain markers, the which are mostly protein. The quantification of these protein markers can be done through western blot, ELISA, enzymatic assays or proteomic studies. The present protocol is focused in the identification of proteins markers from several organelles, by WB, ELISA and enzymatic assays. The enrichment of isolated fractions in endoplasmic reticulum (ER), cytosol, nucleus, mitochondria, plasma membrane (PM), cis-Golgi and trans-Golgi can be assessed by detection of 78 kDa glucose-regulated protein (Grp78), glyceraldehyde 3-phosphate dehydrogenase (Gapdh), histone H3, heat shock protein 60 (Hsp60), flotilin 1, golgin A5 and golgin-97, respectively, by WB, and also by ELISA in case of golgin-97. Enrichment of peroxisomes in fractions collected from sucrose gradients can be assessed by a catalase assay adapted from . This protocol can be applied to any mammalian cell line during subcellular fractionation in order to quantify the enrichment of several organelles in the isolated fractions.

Associated Publications: Pérez-Rodriguez S, Ramírez-Lira MdJ, Wulff T, Voldbor BG, Ramírez OT, Trujillo-Roldán MA, Valdez-Cruz NA (2020) Enrichment of microsomes from Chinese hamster ovary cells by subcellular fractionation for its use in proteomic analysis. PLoS ONE 15(8): e0237930. doi: [10.1371/journal.pone.0237930](https://doi.org/10.1371/journal.pone.0237930)

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