

Resource Summary Report

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Dundee peripheral blood mononuclear cell (PBMC) isolation protocol (from whole blood)

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Protocol Information

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Summary: We describe a fast and efficient peripheral blood mononuclear cell (PBMC) isolation for LRRK2 kinase pathway analysis. Gain of kinase function mutations in the Leucine rich repeat kinase 2 (LRRK2) are associated with causing Parkinson's disease. LRRK2 phosphorylates a subgroup of Rab GTPases and their phosphorylation levels mirror LRRK2 kinase activation status. Here, we describe a facile and robust method for isolating PBMCs by facilitated density gradient centrifugation, subsequent treatment with and without the specific LRRK2 kinase inhibitor MLI-2 to ensure that any effect seen is LRRK2 kinase dependent and cell lysis. PBMC lysates can then be used to quantify LRRK2 kinase pathway activity by measuring LRRK2-mediated phosphorylation of its endogenous RabGTPase substrates in human peripheral blood neutrophils either by quantitative immunoblotting or targeted mass-spectrometry. The benefits of using PBMCs are that they are routinely used as a biomatrix for Parkinson's research and their isolation procedure is significantly faster and easier than that for example of neutrophils or monocytes from peripheral blood. However, their heterogeneity in terms of cellular composition and highly variable expression levels of LRRK2 and its substrates including Rab10 make PBMC a suboptimal biomatrix for reliably measuring LRRK2 kinase pathway activity. A potential use could be target engagement studies in clinical trials targeting LRRK2.

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