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Stimulation of DA neurons and microglia

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We use this protocol and it's working

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Abstract

This procotol describes the details for stimulation of dopaminergic neurons and microglia cultures with IFN-gamma, TNF-alpha and Poly-IC and how to harvest the cells for bulkRNA-seq.

Stimulation

2d

1 Terminally differentiated DA neurons and microglia were stimulated with different compounds diluted in the appropriate culture medium for 48:00:00 at 37 °C

2d

	Compound	Concentration	Provider	Cat# Number
	IFN-gamma	5ng/ml	Miltenyi Biotec	130-096-873
	TNF-alfa	5ng/ml	Miltenyi Biotec	130-094-014
	Poly IC	10ng/ml	Sigma Aldrich	P1530-25MG

Harvesting for bulk-RNAseq

2 Post stimulation, cells were washed twice with 1XPBS and detached with Accutase (Thermo Fisher Scientific: A1110501). Add 500ul/well of accutase and incubate for 7-8min (see under the microscope, if all the cells are detached or leave longer until they detach)

3 Collect samples in the labelled tubes and wash the plate again with PBS to collect remaining cells in the well, and add them to the same tube.

4 Spin down the tube containing samples at 4000 rpm for 5 minutes. Remove the supernatant and place the pellet on dry ice.

5 Cell pellets were frozen down on dry ice and kept at -80 °C for bulk-RNA seq.