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Opentrons Pipeline: DNA Extraction with the Mag-Bind Blood & Tissue DNA HDQ 96 Kit Tissue Protocol V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol is an automated pipeline to extract a full 96-well plate of DNA from tissue lysates.
This protocol was developed and optimized for the following:
Platform: Opentrons OT-2 automated pipetting robot
Kit: Mag-Bind® Blood & Tissue DNA HDQ Prefilled 96 Kit
Recommended number of samples: 96
Two ellutions: Firts ellution(60µL) , Second ellution (30µL)

Samples Lysis

1 Preparate 96 samples lysis following the Mag-Bind ® Blood & Tissue DNA HDQ 96 Kit Tissue Protocol:

1.1 Mince up to 10 mg tissue and transfer to a 1.5 mL tubes (one for each sample).
(Cut each 10 mg sample with sterilized cutters as small as possible)

1.2 Add 250 µL TL Buffer to each sample.

1.3 Add 20 µL Proteinase K Solution to each sample. Vortex to mix.

1.4 Incubate at 55°C overnight in a shaking water bath.

1.5 Centrifuge at maximum speed (≥4,000 x g) for 5 minutes to pellet undigested tissue debris and transfer 200 µL of the supernatant to the VWR 96-Well Deep Well Plate (1000 µL) without
disturbing the undigested pellet

List of materials to start to use OT-2

2 It is necessary to have this equipment to use this pipeline.
Opentrons Equipment List:

	A	B
	OT-2	
	OT-2 8-Channel Pipette P300	
	OT-2 Magnetic Module GEN2	Slot 7

3 Tips & Labware:

	A	B
	Four Opentrons 200µL Filter Tips	Slot: 5,6,8,9
	Two Nest 1-Well Reservoirs (195 mL)	Slot: 10 and 11
	VWR 96-Well Deep Well Plate (1000 µL)	Slot 7 on the Magnetic module
	Two Nest Well plate (100 µL)	Slot 2 and 3
	Two Nest 12 Well Reservoirs (15 mL)	Slot 1 and 4

Star OT-2 run

4 The pipeline start on the step number 7 of the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit Tissue Protocol

Load the pipeline on the Opentrons app: Extracción.py 11KB

The VWR 96-Well Deep Well Plate (1000 µL) labware can be download from: **dx.doi.org/10.17504/protocols.io.dm6gp39njvzp/v1**

4.1 Order of the reagents on the Two Nest 12 Well Reservoirs (15 ml)

Slot 1:

	A	B	C	D	E	F	G	H	I	J	K	L
	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
	11.5 mL of AL buffer	11.5 mL of AL buffer	Mag-Bind mixed with HBQ buffer (20:340) in a total of 12 mL	Mag-Bind mixed with HBQ buffer (20:340) in a total of 12 mL	Mag-Bind mixed with HBQ buffer (20:340) in a total of 12 mL	15 ml of VHB buffer	15 ml of VHB buffer	15 ml of VHB buffer	15 ml of VHB buffer			

Slot 4:

	A	B	C	D	E	F	G	H	I	J	K	L
	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8	Well 9	Well 10	Well 11	Well 12
	15 ml of VHB buffer	15 ml of VHB buffer	15 ml of VHB buffer	15 ml of VHB buffer	15 ml of SPM buffer	15 ml of SPM buffer	15 ml of SPM buffer	15 ml of SPM buffer				Elluti on Buffer