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Working

UC Davis - Inflammation pathway

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Mouse Metabolic Phenotyping Centers
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ABSTRACT

Summary:

This test is designated to determine if rodents exhibit inflammatory disorders, through evaluation of the activation state of key proteins such as JNK and p38 MAP kinases. We will examine the inflammatory state of adipose and liver tissues.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=103&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Cell Lysis Buffer (10X)	9803	Cell Signaling Technology		
4-20% Tris-Glycine Gels	EC60285BOX	Invitrogen - Thermo Fisher		
Tris-Glycine SDS Sample Buffer	LC2676	Invitrogen - Thermo Fisher		
Tris-Glycine SDS Running Buffer	LC26755	Invitrogen - Thermo Fisher		
Tris-Glycine Transfer Buffer	NP00061	Invitrogen - Thermo Fisher		
Methonol	A412P-4	Fisher Scientific		
PVDF 0.2 µm pore size	LC2002	Invitrogen - Thermo Fisher		
WesternBreeze® Chemiluminescent Kit–Anti-Mouse	WB7104	Invitrogen - Thermo Fisher		
WesternBreeze® Chemiluminescent Kit-Anti-Rabbit	WB7106	Invitrogen - Thermo Fisher		
XCell SureLock® Mini-Cell and XCell IITM Blot Module Kit	EI0002	Invitrogen - Thermo Fisher		
Phospho-MAPK Family Antibody Kit	9910	Cell Signaling Technology		AB_330792
MCP-1 Antibody	2029	Cell Signaling Technology		AB_1264199
MCP-5 Antibody	Ab9737	Abcam		AB_308773
TNFalpha Antibody	Ab1793	Abcam		AB_302615
IL-6	Ab6672	Abcam		AB_2127460
Thermo Scientific Pierce* BCA Protein Assay Kits	23225	Thermo Scientific		
Cuvette 1.5ml	14-955-127	Fisher Scientific		
MAPK Family Antibody kit	9926	Cell Signaling Technology		AB_330797

MATERIALS TEXT

Note:

Cell Signaling Technology Pathway Database, RRID:SCR_002071

Fisher Scientific, RRID:SCR_008452

Invitrogen Antibodies, RRID:SCR_008410

Abcam, RRID:SCR_012931

Phospho-MAPK Family Antibody Kit #9910, Cite this, **(Cell Signaling Technology Cat# 9910,** RRID:AB_330792**)**

MAPK Family Antibody kit, Cite this, **(Cell Signaling Technology Cat# 9926,** RRID:AB_330797**)**

MCP-1 Antibody # 2029, Cite this, **(Cell Signaling Technology Cat# 2029,** RRID:AB_1264199**)**

MCP-5 Antibody #Ab9737, Cite this **(Abcam Cat# ab9737,** RRID:AB_308773**)**

TNFalpha Antibody #Ab1793, Cite this, **(Abcam Cat# ab1793,** RRID:AB_302615**)**

IL-6 # Ab6672, Cite this, **(Abcam Cat# ab6672,** RRID:AB_2127460**)**

1 Unless otherwise requested by the PI or stated in the protocol, mice will be euthanized using cervical dislocation.

2 Collect maximum blood from portal vein and isolate plasma according to standard protocols or as desired by the P.I.

3 Quickly collect tissues designated by the P.I. Each tissue should be divided into three portions, one portion should be snap frozen in liquid nitrogen, one portion should be kept into RNA later solution and the third one should be fixed into the appropriate fixative solution. Please note that the whole procedure of tissue collection should be done within 3 minutes maximum.

4 For western blotting, tissues will be lysed into the appropriate lysis buffer.

5 Total protein expression of MCP1/5, IL-6 and TNF-alpha, activation of JNK and p38 in adipose tissue and/or liver (or any other tissue if requested by the P.I.) will be determined according to the standard Western blotting protocols.

6 **Note:**

Evaluation of the activation state of other component of inflammation, such as circulating levels of cytokines is also possible upon special request. Extra charges may apply.

Gene expression of pro-inflammatory cytokines is also soable if requested by the P.I. Extra charges may apply.

Evaluation of the activation state of other component of the ER stress and ER stress-associated signaling, particularly the unfolded protein response (such as ERAD proteins, calnexin, calpains, etc...), JNK pathway or ER stress-induced apoptosis is also possible upon special request. Extra charges may apply.

Immunohistochemistry of inflammation markers such as TNFα and MCP1 couldbe performed on fixed tissues if desired by the P.I. Extra charges may apply.