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UC Davis - Inflammation pathway

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Protocol status: Working

We use this protocol and it's working

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Keywords: Inflammatory disorder, rodents

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.



Materials

MATERIALS

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

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 TNF α and MCP1 couldbe performed on fixed tissues if desired by the P.I. Extra charges may apply.