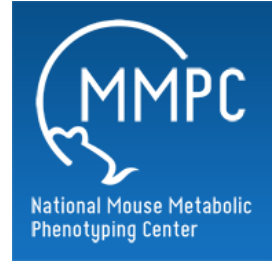


May 13, 2019

## UC Davis - Leptin Signaling pathway

DOI

[dx.doi.org/10.17504/protocols.io.ybqfsmw](https://dx.doi.org/10.17504/protocols.io.ybqfsmw)



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DOI: [dx.doi.org/10.17504/protocols.io.ybqfsmw](https://dx.doi.org/10.17504/protocols.io.ybqfsmw)

External link: <https://mmpc.org/shared/document.aspx?id=247&docType=Protocol>

**Protocol Citation:** Fawaz G. Haj 2019. UC Davis - Leptin Signaling pathway. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.ybqfsmw>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** February 19, 2019

**Last Modified:** May 13, 2019

**Protocol Integer ID:** 20560

**Keywords:** JAK2 and STAT3 proteins, Leptin Signaling pathway

## Abstract

### Summary:

This test is designated to determine if rodents exhibit activation of leptin signaling pathway through evaluation of the phosphorylation state of JAK2 and STAT3 proteins.



## 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-Jak2 Antibody **Cell Signaling Technology Catalog #3771**
- ✕ Jak2 Antibody **Cell Signaling Technology Catalog #4040**
- ✕ Phospho-STAT3 Antibody **Cell Signaling Technology Catalog #9914**
- ✕ STAT3 Antibody **Cell Signaling Technology Catalog #9939**
- ✕ Leptin R Antibody **Abcam Catalog #ab5593**
- ✕ Thermo Scientific Pierce\* BCA Protein Assay Kits **Thermo Scientific Catalog #23225**
- ✕ Cuvette 1.5ml **Fisher Scientific Catalog #14-955-127**

### Note:

**Cell Signaling Technology Pathway Database, [RRID:SCR\\_002071](#)**

**Fisher Scientific, [RRID:SCR\\_008452](#)**

**Abcam, [RRID:SCR\\_012931](#)**

**Phospho-Jak2 Antibody #3771, Cite this, (Cell Signaling Technology Cat# 3771, [RRID:AB\\_330403](#))**

**Jak2 Antibody #4040, Cite this, (Cell Signaling Technology Cat# 4040, [RRID:AB\\_10691469](#))**

**Phospho-Stat Antibody Sampler Kit #9914, Cite this, (Cell Signaling Technology Cat# 9914, [RRID:AB\\_330385](#))**

**STAT3 Antibody #9939, Cite this, (Cell Signaling Technology Cat# 9939, [RRID:AB\\_330426](#))**

**Leptin R Antibody #ab5593, Cite this, (Abcam Cat# ab5593, [RRID:AB\\_304969](#))**

- 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 Leptin Receptor, JAK2, and STAT3, phosphorylation of JAK2 and STAT3 in tissues of interest will be determined according to the standard Western blotting protocols.
- 6 **Note:**

Evaluation of the activation state of other component of the leptin signaling and other associated signaling pathways is also possible upon special request. Extra charges may apply.

Gene expression of proteins involved in leptin signaling is feasible if requested by the P.I. Extra charges may apply.

Immunohistochemistry of leptin signaling could be performed on fixed tissues if desired by the P.I. Extra charges may apply.