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Working

## UC Davis - Leptin Signaling pathway

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### 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.

### EXTERNAL LINK

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

### MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Cell Lysis Buffer (10X)	9803	Cell signaling		
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		
Methanol	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 ITM Blot Module Kit	EI0002	Invitrogen - Thermo Fisher		
Phospho-Jak2 Antibody	3771	Cell Signaling Technology		AB_330403
Jak2 Antibody	4040	Cell Signaling Technology		AB_10691469
Phospho-STAT3 Antibody	9914	Cell Signaling Technology		AB_330385
STAT3 Antibody	9939	Cell Signaling Technology		AB_330426
Leptin R Antibody	ab5593	Abcam		AB_304969
Thermo Scientific Pierce® BCA Protein Assay Kits	23225	Thermo Scientific		
Cuvette 1.5ml	14-955-127	Fisher Scientific		

### MATERIALS TEXT

#### 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.



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