Leptin Signaling pathway

Version: 1
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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Cell Lysis Buffer (10X)	Cell signalling	9803
4-20% Tris-Glycine Gels	Invitrogen	EC60285BOX
Tris-Glycine SDS Sample Buffer	Invitrogen	LC2676
Tris-Glycine SDS Running Buffer	Invitrogen	LC26755
Tris-Glycine Transfer Buffer	Invitrogen	NP00061
Methonol	Fisher Scientific	A412P-4
PVDF, 0.2 µm pore size	Invitrogen	LC2002
WesternBreeze® Chemiluminescent Kit–Anti-Mouse	Invitrogen	WB7104
WesternBreeze® Chemiluminescent Kit-Anti-Rabbit	Invitrogen	WB7106
XCell *SureLock*® Mini-Cell and XCell II™ Blot Module Kit	Invitrogen	EI0002
Phospho-Jak2 Antibody	Cell Signaling	3771
Jak2 Antibody	Cell Signaling	4040
Phospho-STAT3 Antibody	Cell Signaling	9914
STAT3 Antibody	Cell Signaling	9939
Leptin R Antibody	abcam	ab5593
Thermo Scientific Pierce* BCA Protein Assay Kits	Thermo Scientific	23225
Cuvette 1.5ml	Fisher Scientific	14-955-127

Protocol:

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.

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.