Endoplasmic Reticulum Stress

Version: 1
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Summary:

This test is designated to determine if rodents exhibit signs of endoplasmic reticulum stress, through evaluation of the activation state of the 3 sub-arms: PERK/EiF2α, Ire1α/sXBP1, and ATF6α pathways such. We will examine induction of ER stress in adipose and liver tissues.

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
ER Stress Antibody Kit	Cell signaling	9956
Phospho-PERK (Thr980)	Cell signaling	3179
Phospho-EiF2α (Ser51)	Cell signaling	3597
Phospho-Ire1 α (Ser724)	Abcam	Ab48187
ATF6	Abcam	Ab11909
XBP1	Abcam	Ab37152
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 XBP1, BiP, ATF6α, phosphorylation of PERK, Ire1α and EIF2α 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.

Note:

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, etc...), JNK pathway or ER stress-induced apoptosis is also possible upon special request. Extra charges may apply.

Gene expression of proteins involved in unfolded protein response and ER stress is also feasible if requested by the P.I. Extra charges may apply.

Immunohistochemistry of ER stress markers could be performed on fixed tissues if desired by the P.I. Extra charges may apply.