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UC Davis - Endoplasmic reticulum stress

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Protocol status: Working

We use this protocol and it's working

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Abstract

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/EiF2 α , Ire1 α /sXBP1, and ATF6 α pathways such. We will examine induction of ER stress in 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, Replaced by XP04205BOX**
- ✕ 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 0.2um 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 II™ Blot Module Kit **Invitrogen - Thermo Fisher Catalog #EI0002**
- ✕ ER Stress Antibody Kit **Cell Signaling Technology Catalog #9956**
- ✕ Phospho-PERK (Thr980) **Cell Signaling Technology Catalog #3179**
- ✕ Phospho-Eif2 α (Ser51) **Cell Signaling Technology Catalog #3597**
- ✕ Phospho-Ire1 α (Ser724) **Abcam Catalog #Ab48187**
- ✕ ATF6 **Abcam Catalog #Ab11909**
- ✕ XBP1 **Abcam Catalog #Ab37152**
- ✕ 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](#)

Invitrogen Antibodies, [RRID:SCR_008410](#)

ER Stress Antibody Kit #9956, Cite this, (Cell Signaling Technology Cat# 9956, [RRID:AB_823683](#))

Phospho-PERK (Thr980) (16F8) Rabbit mAb #3179, Cite this, (Cell Signaling Technology Cat# 3179, [RRID:AB_2095853](#))



Phospho-Ire1 α (Ser724) # Ab48187, Cite this, (**Abcam Cat# ab48187**, **RRID:AB_873899**)

ATF6 antibody (ab11909), Cite this, (**Abcam Cat# ab11909**, **RRID:AB_298691**)

XBP1 antibody (ab37152), Cite this, (**Abcam Cat# ab37152**, **RRID:AB_778939**)

- 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, ATF6 α , phosphorylation of PERK, Ire1 α and EIF2 α 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 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.