May 14, 2019

Working

UC Davis - Endoplasmic reticulum stress

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dx.doi.org/10.17504/protocols.io.yihfub6

Mouse Metabolic Phenotyping Centers
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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/EiF2α, Ire1α/sXBP1, and ATF6α pathways such. We will examine induction of ER stress in adipose and liver tissues.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=102&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Cell Lysis Buffer (10X)	9803	Cell Signaling Technology		
4-20% Tris-Glycine Gels	EC60285BOX,Replaced byXP04205BOX	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		
Methonol	A412P-4	Fisher Scientific		
PVDF 0.2um 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 II™ Blot Module Kit	EI0002	Invitrogen - Thermo Fisher		
ER Stress Antibody Kit	9956	Cell Signaling Technology		AB_823683
Phospho-PERK (Thr980)	3179	Cell Signaling Technology		AB_2095853
Phospho-EiF2α (Ser51)	3597	Cell Signaling Technology		
Phospho-Ire1 α (Ser724)	Ab48187	Abcam		AB_873899
ATF6	Ab11909	Abcam		AB_298691
XBP1	Ab37152	Abcam		AB_778939
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

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, 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.

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.