UC Davis - Insulin signaling pathway

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Working

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Mouse Metabolic Phenotyping Centers
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ABSTRACT

Summary:

This test is designated to determine defects in the insulin signaling pathway, through evaluation of the activation state of the insulin receptor (IR) and its substrate (IRS1/2), as well as downstream target, mainly Akt and MAP kinases.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=101&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Cell Lysis Buffer (10X)	9803	Cell Signaling Technology		
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		
Methonol	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 IITM Blot Module Kit	EI0002	Invitrogen - Thermo Fisher		
p-IR	07-841	Millipore		AB_568831
Total IR	46-687	Millipore		
Insulin Receptor Substrate Antibody Sampler Kit	3015	Cell Signaling Technology		AB_1196650
p-Akt Antibody	4060	Cell Signaling Technology		AB_2315049
Total Akt Antibody	9272	Cell Signaling Technology		AB_329827
p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Duet	8201	Cell Signaling Technology		AB_10695902
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

Thermo Fisher Scientific, RRID:SCR_008452

P-IR # 07-841, Cite this, **(Millipore Cat# 07-841,** RRID:AB_568831)

Insulin Receptor Substrate Antibody Sampler Kit #3015, Cite this, **(Cell Signaling Technology Cat# 3015,** RRID:AB_1196650**)**

Total Akt Antibody #9272, Cite this **(Cell Signaling Technology Cat# 9272,** RRID:AB_329827**)**

p-Akt Antibody #4060, Cite this **(Cell Signaling Technology Cat# 4060,** RRID:AB_2315049**)**

p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Duet #8201, Cite this, **(Cell Signaling Technology Cat# 8201,** RRID:AB_10695902**)**

1 Fast mice for 16 hours by taking away food the day before (3:00pm)

2 The following day, give the mouse an intraperitoneal injection of insulin (10 U/kg) with a 27 G needle.

3 Use cervical dislocation to euthanize mice.

4 Collect maximum blood from portal vein and isolate plasma according to standard protocols or as desired by the P.I.

5 Quickly collect tissues (Liver, Muscle, Adipose and Pancreas). 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.

6 For western blotting, tissues will be lysed into the appropriate lysis buffer.

7 Overall and site-specific tyrosyl phosphorylation of individual components in insulin signaling such as IR and insulin receptor substrates (IRSs) will be determined by immunoprecipiattaion (IP) then probed with phospho-tyrosyl antibodies according to standard protocols. Tyrosine, Serine and/or threonine phosphorylation of other component of the insulin signaling pathway such as Akt and MAP kinases will be determined also according to the standard Western blotting protocols.

8 **Note:**
Evaluation of the activation state of other component in the insulin signaling pathway is also possible upon special request. Extra charges may apply.