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UC Davis - Insulin signaling pathway

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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 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.

Materials

MATERIALS

- Cell Lysis Buffer (10X) **Cell Signaling Technology Catalog #**9803
- 4-20% Tris-Glycine Gels **Invitrogen - Thermo Fisher Catalog #**EC60285BOX
- 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
- Methonol **Fisher Scientific Catalog #**A412P-4
- PVDF 0.2 µm 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 IITM Blot Module Kit **Invitrogen - Thermo Fisher Catalog #**EI0002
- p-IR **Merck Millipore (EMD Millipore) Catalog #**07-841
- Total IR **Merck Millipore (EMD Millipore) Catalog #**46-687
- Insulin Receptor Substrate Antibody Sampler Kit **Cell Signaling Technology Catalog #**3015
- p-Akt Antibody **Cell Signaling Technology Catalog #**4060
- Total Akt Antibody **Cell Signaling Technology Catalog #**9272
- p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Duet **Cell Signaling Technology Catalog #**8201
- 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

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.