Insulin signaling pathway

Version: 1
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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 MAPkinases.

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
p-IR	Millipore	07-841
Total IR	Millipore	46-687
Insulin Receptor Substrate	Cell signalling	3015

Antibody Sampler Kit		
p-Akt Antibody	Cell signalling	4060
Total Akt Antibody	Cell signalling	9272
p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Duet	Cell signalling	8201
Thermo Scientific	Thermo Scientific Pierce* BCA Protein Assay Kits	23225
Cuvette 1.5ml	Fisher Scientific	14-955-127

Protocol:

1. Fast mice for 16hours 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.

Note:

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