

# ANTIBODY TESTING REPORT

## SUMMARY

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**Antigen:** INSR (Uniprot# P06213)

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**Method tested:** Western Blotting

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**Laboratory ID:** LAB06

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**Project ID:** AR130

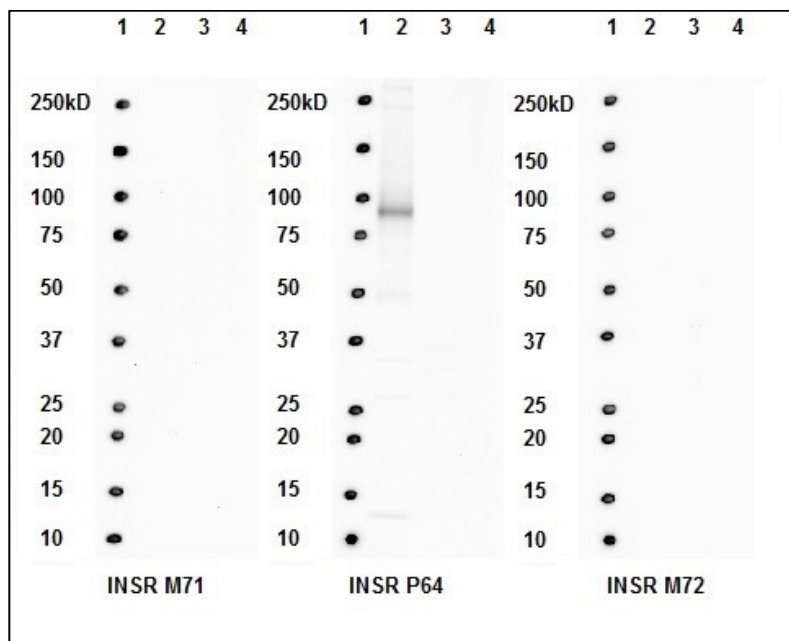
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## BACKGROUND

With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

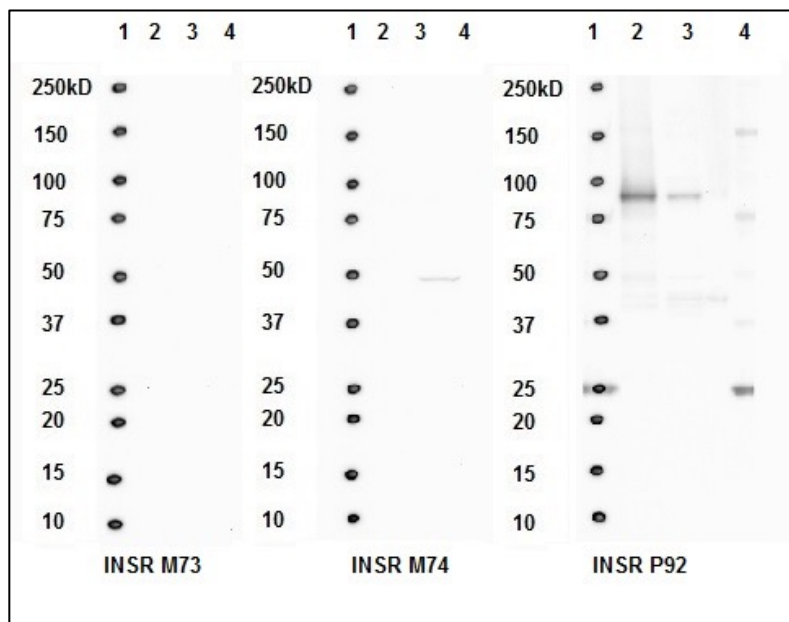
For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

## RESULTS



Western blot analysis of

- (1) MW markers,
- (2) Human kidney tissue lysate,
- (3) Human liver tissue lysate
- (4) HepG2 cell lysate using various anti-INSR antibodies (see Method for primary and secondary antibody details). ECL exposure time was 300 seconds.



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## METHOD

### Antibodies

Primary antibody	Secondary antibody
<b>INSR M71 at 1/1000 (Supplier 25)</b>	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
<b>INSR P64 at 1/1000 (St John's Laboratory)</b>	Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000
<b>INSR M72 at 1/1000 (Supplier 06)</b>	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
<b>INSR M73 at 1/1000 (Supplier 22)</b>	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
<b>INSR M74 at 1/1000 (Supplier 16)</b>	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
<a href="#"><u>INSR P92 at 1/1000 (Santa Cruz)</u></a>	Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000

### Samples

Sample	Description
MW markers (Bio-Rad, Cat no. 161-0376)	Lane 1 - MW markers at 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250kDa.
Human kidney tissue lysate at 25 µg/lane	Lane 2 - Test
Human liver tissue lysate at 25 µg/lane	Lane 3 - Test
HepG2 (Human epithelial cells from liver hepatocellular carcinoma) whole cell lysate at 25 µg/lane	Lane 4 - Test

## PROTOCOL

Western Blotting was performed using BioRad's V3 Workflow System, comprising of a Mini PROTEAN® 3 Dodeca cell, a TransBlot®Turbo™ transfer system and ChemiDoc XRS system.

1. Samples (see table above) were incubated with 4X SDS Sample Buffer at 95-99°C for 3-4 minutes prior to loading. The ratio of samples to sample buffer was adjusted so that the samples contained 2% SDS and 1.25% β-mercaptoethanol.
2. The samples were then loaded and resolved on a on a Criterion™ TGX™ (Tris-Glycine eXtended) precast gel (4-20%) (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by tank transfer and protein transfer was confirmed by using the ChemiDoc XRS imaging system.
4. The immunoblot membrane was blocked in Tris buffered saline (TBS) containing 0.05% Tween-20 (TTBs) and 3% non-fat dry milk powder (blocking buffer) for between 30-45 minutes at room temperature with gentle agitation on a rotary shaker at 100 rpms.
5. The membrane was then immersed with the protein side up in the primary antibody solution (for details see table above) diluted in TTBS containing 1% non-fat dry milk powder for 4 hours at room temperature with gentle agitation.
6. Following a one rinse and three washes for 5 minutes each at room temperature with TTBS, the membrane was incubated in the secondary antibody (for details see table above) diluted in TTBS containing 1% non-fat dry milk for 45 minutes at room temperature with gentle agitation.
7. The membrane was then rinsed once and washed twice with TTBS for 2 minutes and then 4 minutes respectively at room temperature. A final wash in TBS only at room temperature for 5 minutes was then performed.
8. After draining away excess TBS, the membrane was incubated for 2 minutes at room temperature with HRP substrate reagent (prepared just prior to use). Signals were detected using the ChemiDoc XRS imaging system.

## EXPERIMENTAL NOTES

Under these experimental conditions, INSR P92 exhibits immunoreactive bands in the Human tissue lysates and the Human cell line, with a major band at approximately MW of 90kDa shown in the tissue lysates and bands at around 150, 75 and 25kDa in the HepG2 cell line lysate. INSR P64 also exhibits the major 90kDa band in the Human kidney tissue lysate but not in the liver tissue or HepG2 lysates. INSR M71, INSR M72, INSR M73 and INSR M74 do not demonstrate immunoreactivity in this experiment.