ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: IL6 (Uniprot# P05231)

Method tested: Western Blotting

Laboratory ID: LAB06

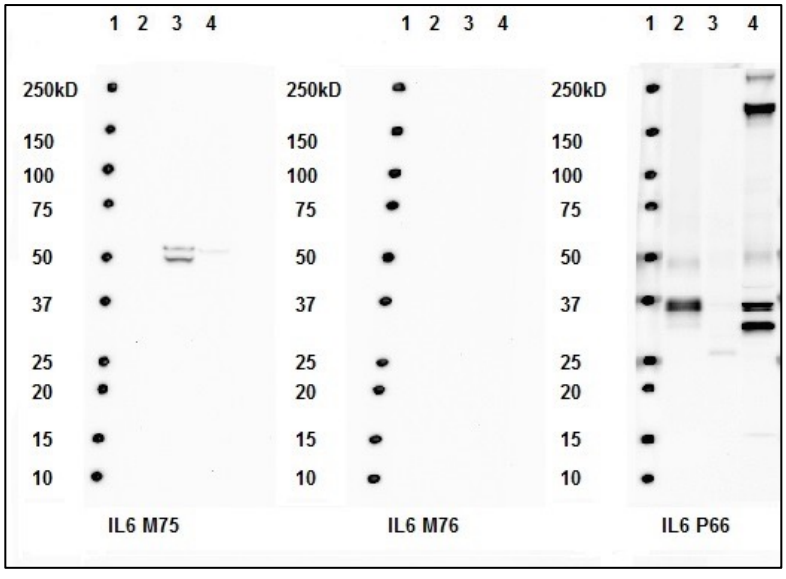
Project ID: AR129

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



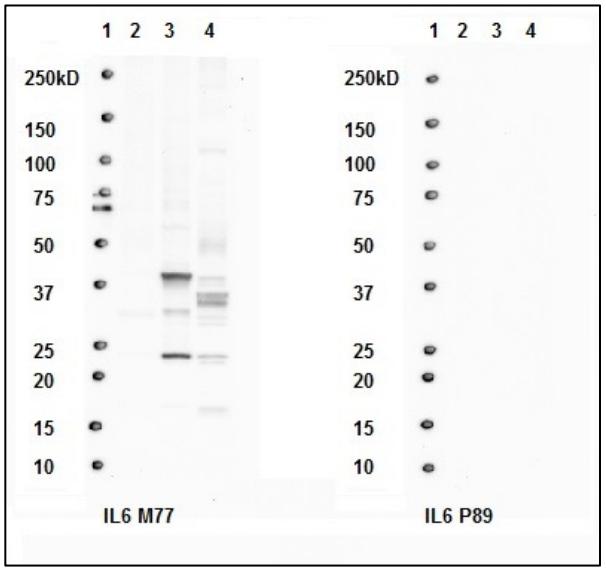
Western blot analysis of

(1) MW markers,

(2) Human lung tissue lysate,

(3) Human liver tissue lysate

(4) Smooth muscle tissue lysate using various anti-IL6 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
	IL6 M75 at 1/1000 (Absea)	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
	IL6 M76 at 1/1000 (Supplier 05)	Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000
	IL6 P66 at 1/1000 (Boster)	Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000
	IL6 M77 at 1/1000 (Fitzgerald)	Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000
	**IL6 P89 at 1/1000 (R & D Systems)**	Mouse anti-Goat IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 205-035-108) at 1/10,000

 = Component of the IL6 Superstarter Antibody Panel. See end of report for details.

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 lung tissue lysate at 25 µg/lane	Lane 2 - Test
Human liver tissue lysate at 25 µg/lane	Lane 3 - Test
Human smooth muscle tissue 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 95-99ºC 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, IL6 M77 detected a band at the expected MW (~24kDa) in the Human liver tissue and smooth muscle tissue lysates along with other bands at various MWs. There was a weak signal observed with the Human lung tissue lysate with a suggestion of a band at ~ 30kDa. No immunoreactivity was seen using either IL6 M76 or IL6 P89.

IL6 P66 and IL6 M75 demonstrated immunoreactivity with bands in some of the Human tissue lysates. These bands may represent isoforms or bound forms of IL6 or they may be non-specific background as the banding pattern varied depending on the antibody used and were inconsistent with the expected MW.

SUPERSTARTER ANTIBODY PANELS

A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only $287, €267, £198.

The IL6 Superstarter Antibody Panel consists of:
-1x ab9770 Abcam (high ratings)
-1x MAB406 R&D Systems (high ratings)
-1x sc-1265 Santa Cruz Biotechnology (star performer)
http://www.antibodyresource.com/superstars

Images of Superstar IL6 antibodies: