ANTIBODY TESTING REPORT

SUMMARY

Antigen: TNFα (Uniprot# P01375)

Method tested: Flow Cytometry

**Laboratory ID:** LAB07

Project ID: AR134

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.

Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.
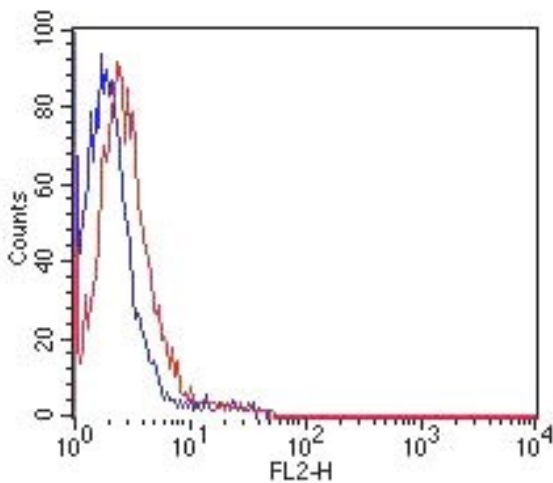
RESULTS

Flow cytometric analysis of paraformaldehyde fixed, THP-1 cells (Human monocytic leukemia cells) using various anti-TNF-α antibodies (red) and isotype controls (blue) (see Method section for more detail).
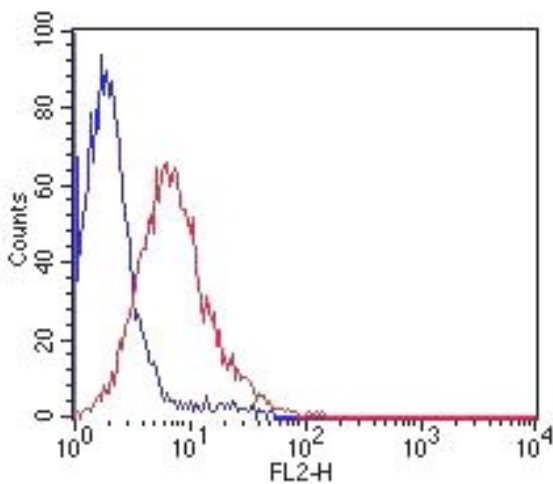
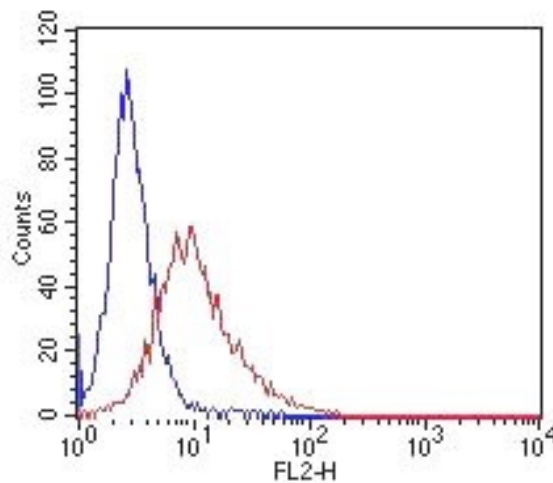Antibody : TNF-α M18 (red)
Isotype control : Mouse IgG (blue)
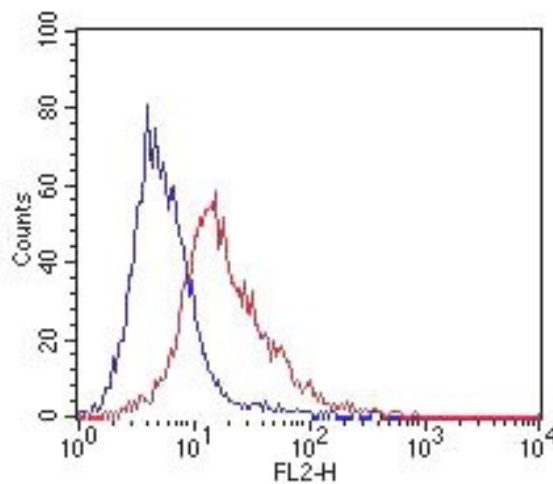
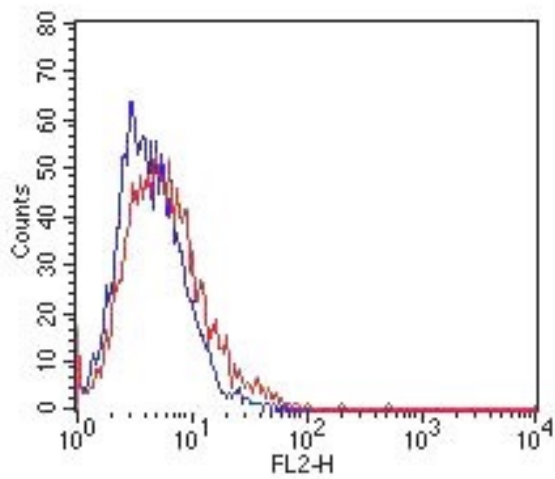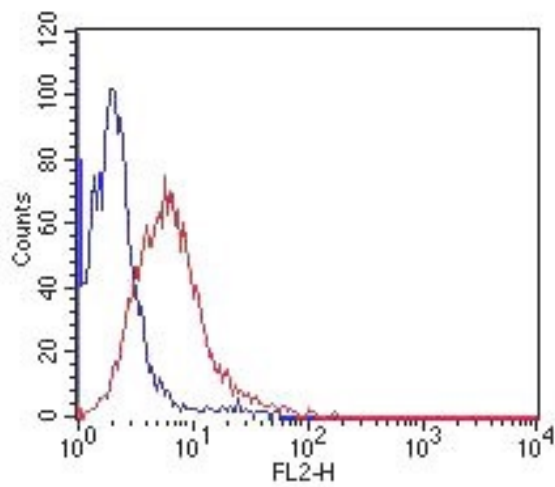Antibody : TNF-α P20 (red)
Isotype control : Goat IgG (blue)
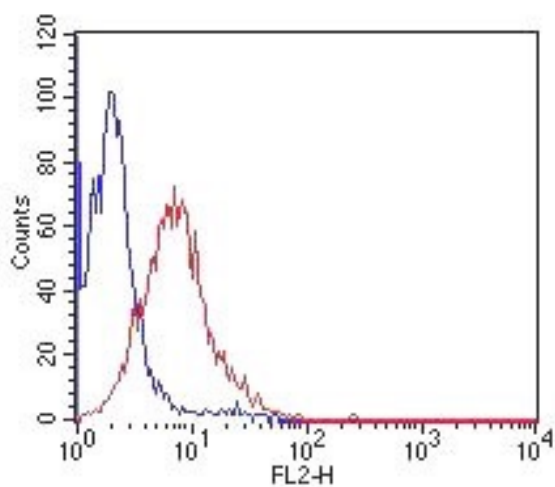

Antibody : TNF-α M19 (red)
Isotype control : Mouse IgG (blue)

Antibody : TNF-α M21 (red)
Isotype control : Mouse IgG2a (blue)

Antibody : TNF-α P28 (red)
Isotype control : Rabbit IgG (blue)

Antibody : TNF-α M86 (red)
Isotype control : Mouse IgG1 (blue)

Antibody : TNF-α M104 (red)
Isotype control : Mouse IgG1 (blue)

METHOD

Antibodies

	Primary antibody	Secondary antibody	Isotype Control
	TNF-α M18 at 2 µg per test (St John's Laboratory)	R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150	Mouse IgG Isotype Control (Thermo Scientific, 10400C) at 2 µg per test
	TNF-α P20 at 2 µg per test (Acris)	R-Phycoerythrin AffiniPure Donkey Anti-Goat IgG (H +L) (Jackson ImmunoResearch, 705-115-147) at 1/150	Goat IgG Isotype Control (Thermo Scientific, 02-6202) at 2 µg per test
	TNF-α M19 at 2 µg per test (Supplier 22)	R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150	Mouse IgG Isotype Control (Thermo Scientific, 10400C) at 2 µg per test
	TNF-α M21 at 2 µg per test (Absea)	R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150	Mouse IgG2a Isotype Control (Thermo Scientific, MA5-14441) at 2 µg per test
	TNF-α P28 at 2 µg per test (Supplier 11)	R-Phycoerythrin AffiniPure Fab fragment Goat Anti-Rabbit, Fc fragment specific (Jackson ImmunoResearch, 111-117-008) at 1/150	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 2 µg per test
	TNF-α M86 at 2 µg per test (HyTest)	R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 2 µg per test
⭐	**TNF-α M104 at 1/10 (abcam)**	R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/10

⭐ = Component of the TNF-α Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Flow Cytometry was performed using a BD FACSCalibur™ platform. Cells were prepared prior to analysis as follows:-

1. Cells, grown in petri dishs, were suspended in cell culture medium, transferred to a 1.5 ml EP tube and the cell concentration adjusted to between 1 and 5×10^6 cells/ml.

2. Following centrifugation at 1700 rpm for 5 minutes and after removal of the supernatant, the cell pellet was washed by adding 8 ml PBS and gentle vortexing. The cell suspension was then centrifuged at 1700 rpm for 5 minutes and the supernatant removed.

3. The cells were fixed in 2% paraformaldehyde by adding 6ml of the paraformaldehyde solution for 10 minutes at room temperature. The cells were then centrifuged and washed as described in step 2.

4. Penetration of the cells was performed by adding 6 ml of precooled methanol, gentle vortexing and incubation for 10 minutes at -20°C. NB. If extracellular staining was required, this step was omitted.

5. Following washing with PBS as described above in step 2, a blocking step was performed by adding 1 ml of 2% BSA in PBS to the cell pellet for 30 minutes at room temperature.

6. The resulting cell suspension was then aliquotted into prelabelled tubes so that 1 ml of cell suspension was available for each of the antibodies or controls to be tested. Following centrifugation of the cell suspension aliquots at 1700 rpm for 5 minutes and removal of each supernatant, the cell pellets were incubated with 0.1 ml of the appropriate primary antibody or control diluted in PBS (for details see table above) for 60 minutes at 37°C.

7. The cells were then washed twice by adding 1 ml of PBS, centrifugation at 1700 rpm for 5 minutes and removal of the supernatants. The resulting cell pellets were incubated with 0.1 ml of the appropriate secondary antibody diluted in PBS (for details see table above) for 40 minutes at 37°C and protected from light.

8. Following this incubation, the cells were washed twice with PBS as described in step 7 and the resulting cell pellets resuspended in 0.2 ml PBS in preparation for flow cytometric analysis.

EXPERIMENTAL NOTES

Under these experimental conditions, TNF-α M18, TNF-α M21, TNF-α M86, TNF-α M104 and TNF-α P20 demonstrated positive staining of the THP-1 cells whilst negative staining was observed using TNF-α M19 and TNF-α P28.

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 $291, €267, £198.

The TNF-α Superstarter Antibody Panel consists of:
- 1x Santa Cruz Biotechnology sc-133192 (high ratings)
- 1x Abcam ab6671 (high ratings)
- 1x R&D Systems MAB210 (high ratings)
http://www.antibodyresource.com/superstars

Images of Superstar TNF-α antibodies: