# ANTIBODY TESTING REPORT

## SUMMARY

**Antigen:** CD86 (Uniprot# P42081)

**Method tested:** Flow Cytometry

**Laboratory ID:** LAB07

**Project ID:** AR149

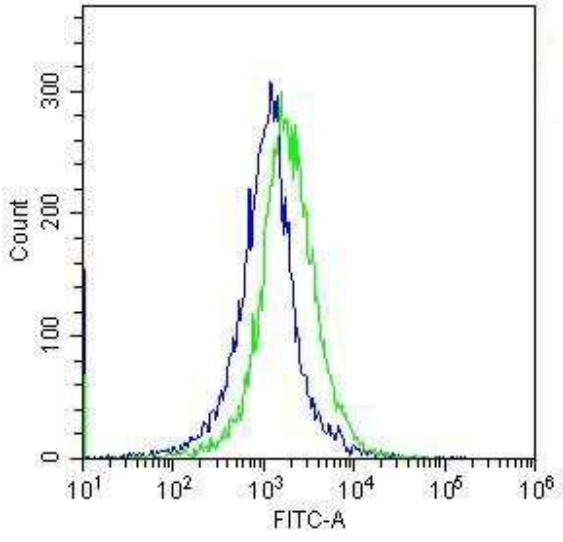
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical 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.

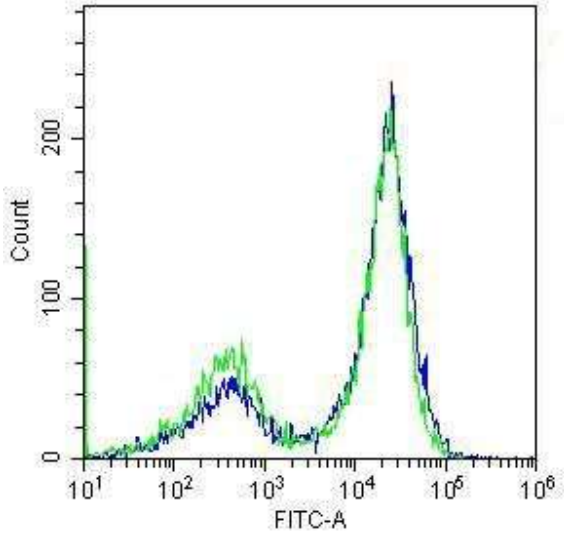
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

## RESULTS

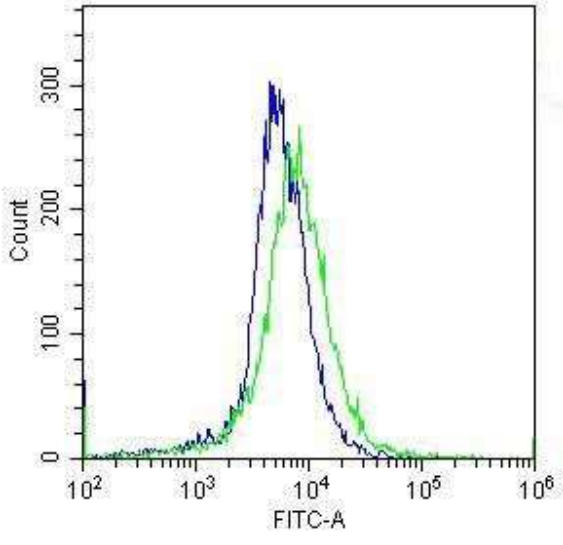
Flow cytometric analysis of formaldehyde fixed, Raji cells (Human Burkitt's leukemia cells) and non-fixed Mouse C57BL/6 splenocytes using various anti-CD86 antibodies (green) and isotype controls (blue) (see Method section for more detail).



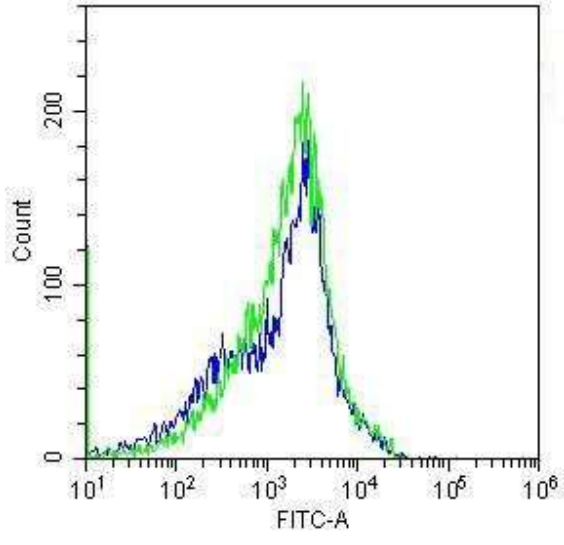

Cells : Raji
Antibody : CD86 M161 (green)
Isotype control : Mouse IgG1 (blue)



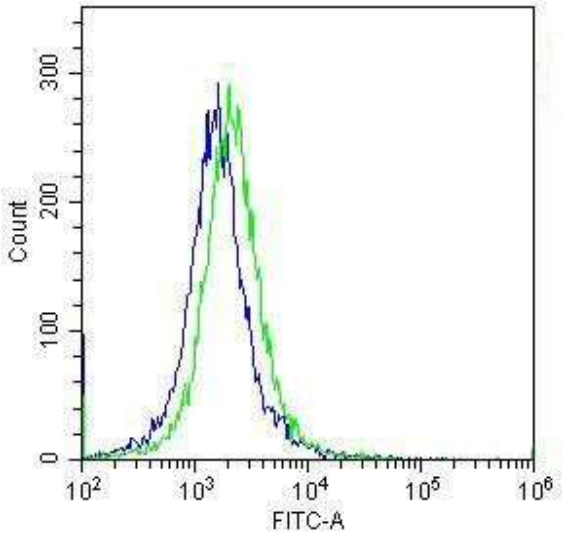

Cells : Mouse splenocytes
Antibody : CD86 M161 (green)
Isotype control : Mouse IgG1 (blue)



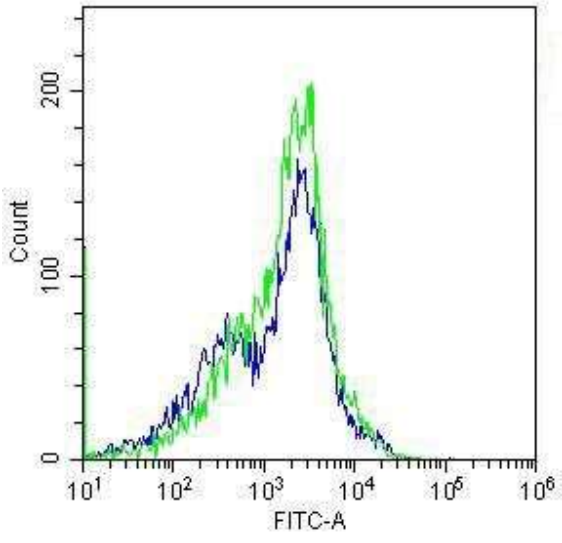

Cells : Raji
Antibody : CD86 M165 (green)
Isotype control : Rat IgG2a (blue)



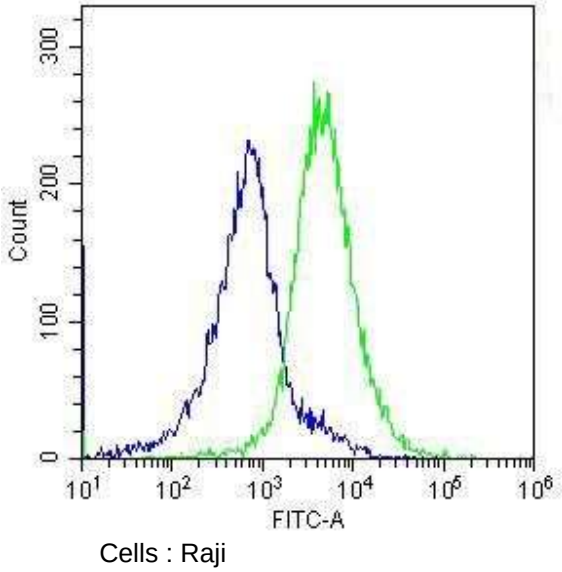

Cells : Mouse splenocytes
Antibody : CD86 M165 (green)
Isotype control : Rat IgG2a (blue)



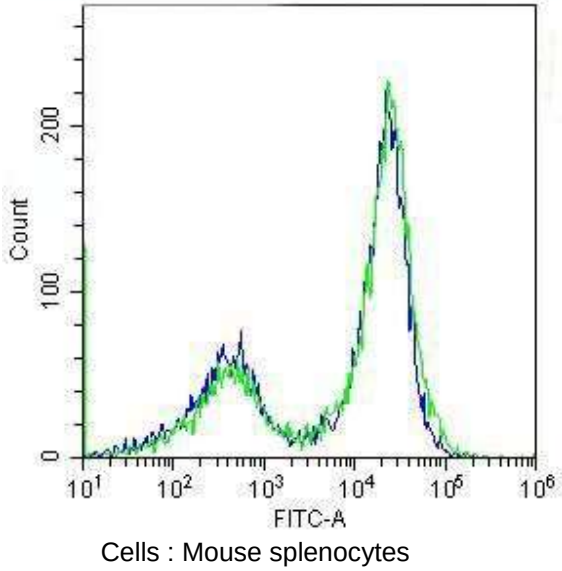

Cells : Raji
Antibody : CD86 M166 (green)
Isotype control : Rat IgG2b (blue)



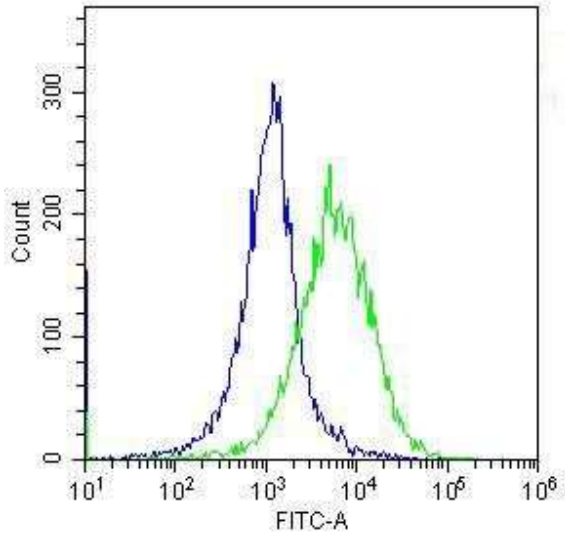

Cells : Mouse splenocytes
Antibody : CD86 M166 (green)
Isotype control : Rat IgG2b (blue)



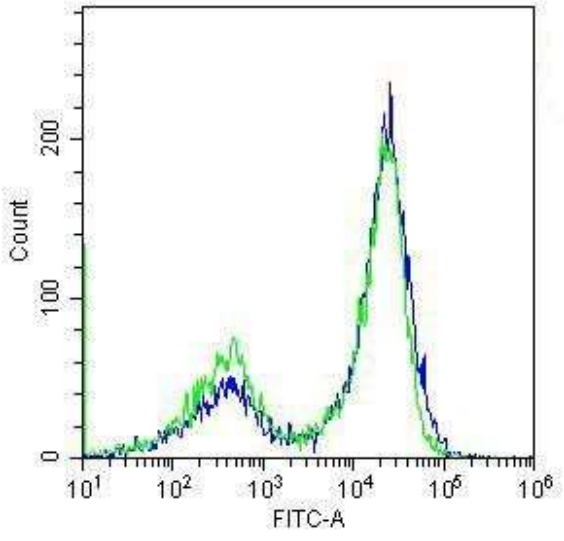

Cells : Raji
Antibody : CD86 M167 (green)
Isotype control : Mouse IgG2b (blue)




Cells : Mouse splenocytes
Antibody : CD86 M167 (green)
Isotype control : Mouse IgG2b (blue)




Cells : Raji
Antibody : CD86 M187 (green)
Isotype control : Mouse IgG1 (blue)




Cells : Mouse splenocytes
Antibody : CD86 M187 (green)
Isotype control : Mouse IgG1 (blue)

# METHOD

## Antibodies

| | Primary antibody | Secondary antibody | Isotype Control |
|---|---|---|---|
| | **CD86 M161 (Supplier 40)** | Donkey anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (ThermoFisher Scientific, A-21202) | Mouse IgG1 Isotype Control (ThermoFisher Scientific, MA5-14453) |
| | **CD86 M165 (Supplier 37)** | Alexa Fluor® 488 AffiniPure Donkey Anti-Rat IgG (H+L) (Jackson ImmunoResearch, 712-545-150) | Rat IgG2a Isotype Control (ThermoFisher Scientific, PA5-33214) |
| | **CD86 M166 (Supplier 37)** | Alexa Fluor® 488 AffiniPure Donkey Anti-Rat IgG (H+L) (Jackson ImmunoResearch, 712-545-150) | Rat IgG2b Isotype Control (ThermoFisher Scientific, MA1-90911) |
| | CD86 M167 (Biolegend) | Donkey anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (ThermoFisher Scientific, A-21202) | Mouse IgG2b Isotype Control (ThermoFisher Scientific, MA5-14447) |
| | CD86 M187 (Invitrogen) | Donkey anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (ThermoFisher Scientific, A-21202) | Mouse IgG1 Isotype Control (ThermoFisher Scientific, MA5-14453) |

= Component of the CD86 Superstarter Antibody Panel. See end of report for details.

All primary antibodies and Isotype Controls were tested at 0.5 µg/$10^6$ cells in 100 µl and secondary antibodies at 1/200.

# PROTOCOL

Flow Cytometry was performed using a Beckman CytoFlex platform.

Raji cells were prepared prior to analysis as follows:-

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

Mouse C57BL/6 splenocytes were prepared prior to analysis as follows:-

1. Spleens were isolated by dissection from sacrificed C57BL/6 mice.
2. Cells were collected by washing the spleens out with PBS from a 1ml syringe. Following filtration through a 70 µm aperture cell strainer to remove any debris, PBS was added to a volume of 2ml.
3. After gentle vortexing, the cell suspension was centrifugated at 1400 rpm for 5 minutes and then the supernatant discarded.
4. Following a further wash with PBS as described in step 3, lysis of erythrocytes was performed by adding 2 ml of Lysing Solution (Beckman, A09777) and incubation of the cell suspension for 10 minutes at room temperature, protected from light.
5. The cells were then washed twice by adding 2 ml of PBS, centrifugation at 1400 rpm for 5 minutes and removal of the supernatants.
6. PBS was added to the cells so that the cell concentration adjusted to between 1 and 5 x $10^6$ cells/ml.
7. 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.
8. Steps 6 – 8 from above, as described for Raji cell incubation with primary and secondary antibodies, were then followed.

## EXPERIMENTAL NOTES

Under these experimental conditions, CD86 M167 and CD86 M187 demonstrated positive staining of the Raji cells but not the Mouse splenocytes. For both cell types, no binding was shown with any of the other antibodies tested.

# 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 $271, €236, £175.

The CD86 Superstarter Antibody Panel consists of:

| | | |
|---|---|---|
| 1 x | sc-19617 | (Santa Cruz Biotechnology) |
| 1 x | 305406 | (BioLegend) |
| 1 x | 555658 | (BD Biosciences) |

http://www.antibodyresource.com/superstars

Images of Superstar CD86 antibodies: