SUMMARY

Antigen: CD4 (Uniprot# P01730)

Method tested: Flow Cytometry

Laboratory ID: LAB07

Project ID: AR135

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 leukaemia cells) using various anti-CD4 antibodies (red) and isotype controls (blue) (see Method section for more detail).

Antibody: CD4 M56 (red)
Isotype control: Mouse IgG1 (blue)

Antibody: CD4 M57 (red)
Isotype control: Mouse IgG2b (blue)

Antibody: CD4 M58 (red)
Isotype control: Mouse IgG (blue)

Antibody: CD4 P59 (red)
Isotype control: Goat IgG (blue)
Antibody : CD4 M59 (red)
Isotype control : Mouse IgG1 (blue)

Antibody : CD4 M60 (red)
Isotype control : Mouse IgG1 (blue)

Antibody : CD4 M61 (red)
Isotype control : Mouse IgG1 (blue)

Antibody : CD4 M100 (red)
Isotype control : Mouse IgG1 (blue)
### METHOD

#### Antibodies

<table>
<thead>
<tr>
<th>Primary antibody</th>
<th>Secondary antibody</th>
<th>Isotype Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD4 M56 at 1/1000 (Supplier 15)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/1000</td>
</tr>
<tr>
<td><strong>CD4 M57 at 1/1000 (St John’s Laboratory)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG2b Isotype Control (Thermo Scientific, MA5-14447) at 1/1000</td>
</tr>
<tr>
<td><strong>CD4 M58 at 1/1000 (Supplier 29)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG Isotype Control (Thermo Scientific, 10400C) at 1/1000</td>
</tr>
<tr>
<td><strong>CD4 P59 at 1/1000 (Supplier 06)</strong></td>
<td>R-Phycoerythrin AffiniPure Donkey Anti-Goat IgG (H +L) (Jackson ImmunoResearch, 705-115-147) at 1/150</td>
<td>Goat IgG Isotype Control (Thermo Scientific, 02-6202) at 1/1000</td>
</tr>
<tr>
<td><strong>CD4 M59 at 1/4000 (Novus)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/4000</td>
</tr>
<tr>
<td><strong>CD4 M60 at 1/1000 (Supplier 11)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/1000</td>
</tr>
<tr>
<td><strong>CD4 M61 at 1/4000 (Supplier 16)</strong></td>
<td>R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/150</td>
<td>Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/4000</td>
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</table>
Flow Cytometry was performed using a BD FACSCalibur™ platform. 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.
EXPERIMENTAL NOTES
Under these experimental conditions, CD4 M57, CD4 M59 and CD4 M100 demonstrated very weak binding to the THP-1 cells.

SUPERSTARTER ANTIBODY PANELS
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- 1x BD Biosciences 553729 (star performer)
- 1x BioLegend 100406 (high reviews)
- 1x eBioscience 11-0041-81 (high reviews)

http://www.antibodyresource.com/superstars

Images of Superstar CD4 antibodies: