ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: CD34 (Uniprot# P28906)

Method tested: Flow Cytometry

Laboratory ID: LAB07

Project ID: AR133

BACKGROUND

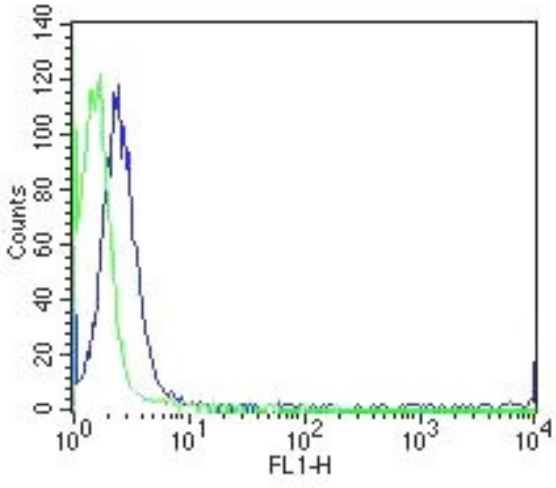
With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical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

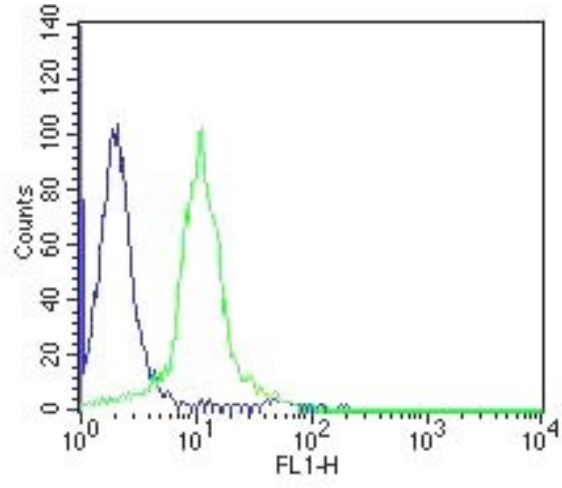
Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

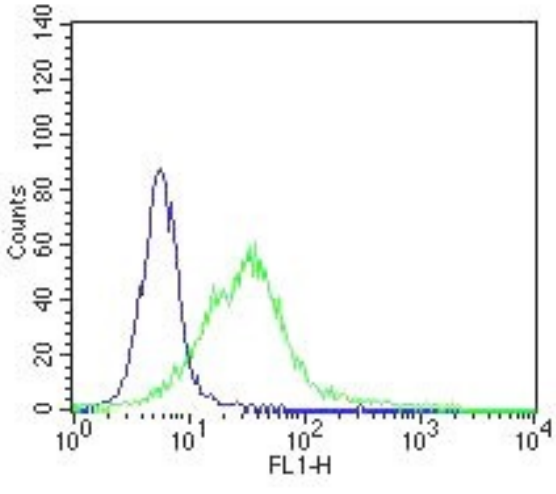
Flow cytometric analysis of paraformaldehyde fixed, Jurkat cells (Human T cell leukaemia cells) using various anti-CD34 antibodies (green) and isotype controls (blue) (see Method section for more detail).



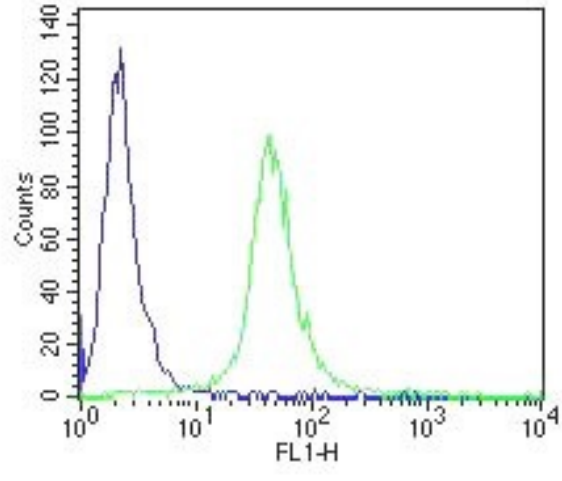
Antibody : CD34 M35 (green)
Isotype control : Mouse IgG1 (blue)



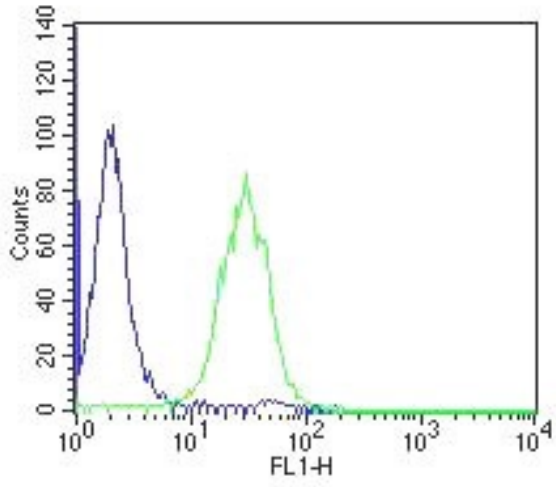
Antibody : CD34 P35 (green)
Isotype control : Rabbit IgG (blue)



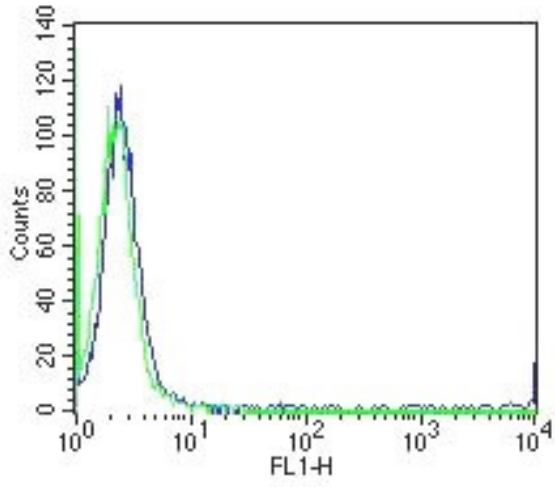
Antibody : CD34 M36 (green)
Isotype control : Rat IgG2a (blue)



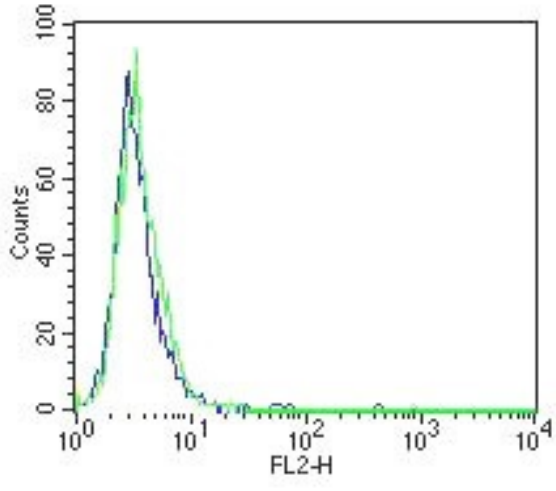
Antibody : CD34 P36 (green)
Isotype control : Goat IgG (blue)



Antibody : CD34 P37 (green)
Isotype control : Rabbit IgG (blue)



Antibody : CD34 M37 (green)
Isotype control : Mouse IgG1 (blue)



Antibody : CD34 M97 (green)
Isotype control : Mouse IgG1 (blue)

METHOD

Antibodies

	Primary antibody	Secondary antibody	Isotype Control
	CD34 M35 at 1/500 (Supplier 15)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/500
	CD34 P35 at 1/50 (St John's Laboratory)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35553) at 1/200	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 1/50
	CD34 M36 at 1/1000 (Novus)	Goat anti-Rat IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, SA5-10018) at 1/200	Rat IgG2a Isotype Control (Thermo Scientific, PA5-33214) at 1/1000
	CD34 P36 at 1/50 (Everest)	Alexa Fluor® 488 AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch, 805-545-180) at 1/200	Goat IgG Isotype Control (Thermo Scientific, 02-6202) at 1/50
	CD34 P37 at 1/50 (Boster)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35553) at 1/200	Rabbit IgG Isotype Control (Thermo Scientific, MA5-16385) at 1/50
	CD34 M37 at 1/50 (Supplier 16)	Goat anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate (Thermo Scientific, 35503) at 1/200	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 1/50

	CD34 M97 at 20µl/test (BD Biosciences)	Not required as primary conjugated to PE	Mouse IgG1 Isotype Control (Thermo Scientific, MA5-14453) at 20µl/test followed by R-Phycoerythrin AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-115-164) at 1/200

= Component of the CD34 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 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^{o}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^{o}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^{o}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, CD34 M36, CD34 P35, CD34 P36 and CD34 P37 demonstrated positive staining of Jurkat cells whilst for all the other tested antibodies negative staining was observed.

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 $202, €176, £130.

The CD34 Superstarter Antibody Panel consists of:
-1x Miltenyi Biotec 130-081-001
-1x R&D Systems AF4117 (high ratings)
-1x Abcam ab8158 (high ratings)
-1x BD Biosciences 555822 (star performer)
http://www.antibodyresource.com/superstars

Images of Superstar CD34 antibodies: