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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	1 mL monoclonal Anti-DLL4 antibodies, human conjugated to:										
	<table border="0"> <tr> <td>FITC</td> <td>130-096-674</td> </tr> <tr> <td>PE</td> <td>130-096-567</td> </tr> <tr> <td>APC</td> <td>130-096-560</td> </tr> <tr> <td>VioBlue®</td> <td>130-096-679</td> </tr> <tr> <td>Biotin</td> <td>130-096-564</td> </tr> </table>	FITC	130-096-674	PE	130-096-567	APC	130-096-560	VioBlue®	130-096-679	Biotin	130-096-564
FITC	130-096-674										
PE	130-096-567										
APC	130-096-560										
VioBlue®	130-096-679										
Biotin	130-096-564										
Clone	MHD4-46 (isotype: mouse IgG1).										
Capacity	100 tests or up to 10 ⁹ total cells.										
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.										
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.										

1.1 Background information

- Antigen: DLL4
- Expression patterns: The antibody MHD4-46 reacts with the human delta-like4 protein (DLL4). DLL4 is a type I membrane protein belonging to the Delta/Serrate/Lag2 (DSL) family of Notch ligands. Notch signaling controls cell fate decisions and is involved in multiple developmental processes. Notch ligands are categorized into two subfamilies: the Jagged and the Delta ligand family. In mammals, five ligands (DLL 1, 3, and 4, Jagged 1 and 2) have been identified. DLL4 is highly expressed within vascular endothelial cells, thymic epithelial cells and dendritic cell subsets and plays an important role for

vascular development and homeostasis^{1,2} and thymic^{3,4} as well as peripheral T cell differentiation⁵.

1.2 Applications

- Identification and enumeration of DLL4⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-DLL4 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

The antibody is suited for staining of formaldehyd-fixed cells.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Anti-DLL4-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

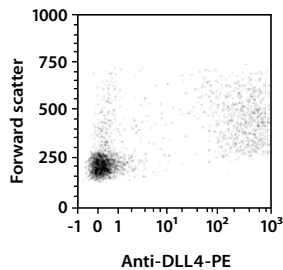
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-DLL4 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. (Optional) If Anti-DLL4-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with Anti-DLL4 antibodies

Delta-like 4 transfected CHO cells mixed with human peripheral blood mononuclear cells (PBMCs) were stained with delta-like 4 antibodies conjugated to PE and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Radtke, F. *et al.* (2010) Notch signaling in the immune system. *Immunity* 32 (1): 14–27.
2. Noguera-Troise, I. *et al.* (2006) Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature* 444 (7122): 1032–1037.
3. Ridgeway, J. *et al.* (2006) Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature* 444 (7122): 1083–1087.
4. Hozumi, K. *et al.* (2008) Delta-like 4 is indispensable in thymic environment specific for T cell development. *J. Exp. Med.* 205 (11): 2507–2513.
5. Koch, U. *et al.* (2008) Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment. *J. Exp. Med.* 205 (311): 2515–2523.

All protocols and data sheets are available at www.miltenyibiotec.com.

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