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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.**

<b>Components</b>	1 mL monoclonal Anti-Jagged2 antibodies, human conjugated to:
	PE 130-097-002
	APC 130-097-004
	Biotin 130-097-008
<b>Clone</b>	MHJ2-523 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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: Jagged2
- Expression patterns: The MHJ2-523 antibody reacts with the Jagged2 protein which is one of the several mammalian Notch ligands. Jagged2 is expressed by various organs and cell types and is involved in regulation of cell differentiation processes, such as lymphocyte development, myogenesis, neurogenesis. Jagged2 is reported to be essential for the regulation of spermatogenesis. Jagged2 is also expressed on some lymphoma and leukemia cells and various other tumor cells. Jagged2 can be expressed by various antigen-presenting cells and may contribute to APC-directed peripheral T cell differentiation.

### 1.2 Applications

- Identification and enumeration of Jagged2<sup>+</sup> cells by flow cytometry.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Jagged2 conjugates is **1:11 for up to 10<sup>7</sup> 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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> 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-Jagged2-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to [www.miltenyibiotec.com/antibodies](http://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 10<sup>7</sup>** nucleated cells. When working with fewer than 10<sup>7</sup> 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 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

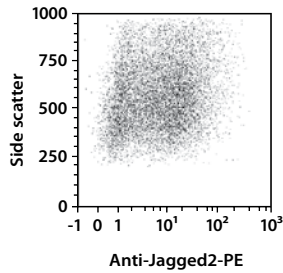
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-Jagged2 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (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-Jagged2-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody,

and continue as described in steps 5 and 6.

- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

### 3. Example of immunofluorescent staining with Anti-Jagged2 antibodies

Human Jagged2-transfected CHO cells were stained with Anti-Jagged2 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](http://www.miltenyibiotec.com/antibodies).

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols.

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