



Miltenyi Biotec

# CD265 (RANK) antibodies, mouse

## For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of  $10^6$  cells.

Product	Content	Order no.
CD265 (RANK)-Biotin	150 µg in 1 mL	130-115-990
CD265 (RANK)-FITC	30 µg in 200 µL	130-116-066
CD265 (RANK)-FITC	150 µg in 1 mL	130-115-991
CD265 (RANK)-PE	30 µg in 200 µL	130-116-067
CD265 (RANK)-APC	30 µg in 200 µL	130-116-068
CD265 (RANK)-APC	150 µg in 1 mL	130-115-993
CD265 (RANK)-Biotin	30 µg in 200 µL	130-116-065

## 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.

## Technical data and background information

<b>Antigen</b>	CD265 (RANK)
<b>Clone</b>	REA961
<b>Isotype</b>	recombinant human IgG1
<b>Isotype control</b>	REA Control antibodies
<b>Alternative names of antigen</b>	RANK, TRANCE-R, TNFRSF11A
<b>Entrez Gene ID</b>	<a href="#">21934</a>
<b>Molecular mass of antigen [kDa]</b>	63
<b>Distribution of antigen</b>	dendritic cells, thymocytes, liver, pancreas, prostate, colon, epithelial cells, other
<b>Product format</b>	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

Clone REA961 recognizes the mouse CD265 antigen, also known as receptor activator of NF-κB (RANK) or tumor necrosis factor receptor superfamily member 11A (TNFRSF11A). RANK is the receptor for RANK-ligand (RANKL) which promotes osteoclast differentiation and activation. CD265 is also expressed on dendritic cells, skeletal muscle, thymus, liver, colon, small intestine, adrenal gland, mammary gland epithelial cells, prostate, vascular cells, and pancreas. Additional information: Clone REA961 displays negligible binding to Fc receptors.

## 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) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

## Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10<sup>6</sup> cells/100 µL.
- Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

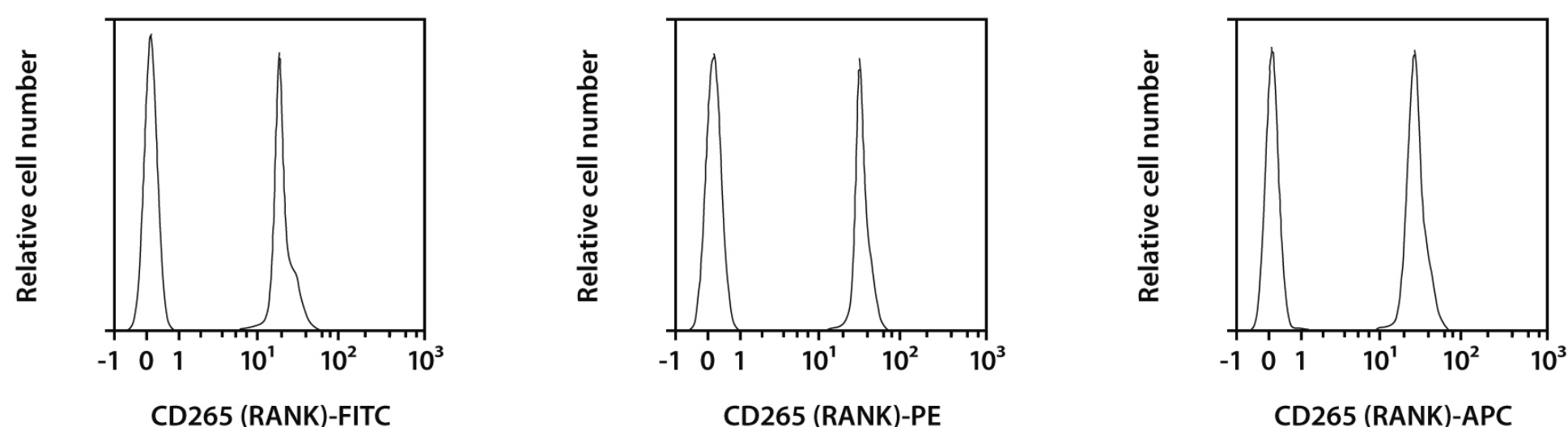
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>6</sup> nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

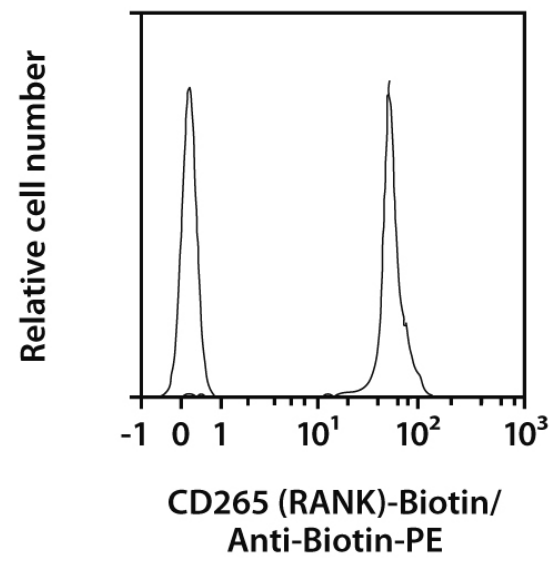
Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## Examples of immunofluorescent staining

Latex beads were coated with recombinant mouse CD265 (RANK) protein and stained with CD265 (RANK) antibodies or with the corresponding REA Control antibodies (left peak). Flow cytometry was performed using the MACSQuant<sup>®</sup> Analyzer.





## Warranty

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