# Anti-EGR-2 antibodies, mouse

## For research use only



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

| Product | Content | Order no. |
|---|---|---|
| Anti-EGR-2-FITC | 150 µg in 1 mL | 130-114-255 |
| Anti-EGR-2-FITC | 30 µg in 200 µL | 130-114-362 |
| Anti-EGR-2-PE | 30 µg in 200 µL | 130-114-363 |
| Anti-EGR-2-PE | 150 µg in 1 mL | 130-114-256 |
| Anti-EGR-2-APC | 30 µg in 200 µL | 130-114-364 |
| Anti-EGR-2-APC | 150 µg in 1 mL | 130-114-257 |
| Anti-EGR-2-Vio515 | 30 µg in 200 µL | 130-114-365 |
| Anti-EGR-2-Vio515 | 150 µg in 1 mL | 130-114-258 |

## 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

| | |
|---|---|
| **Antigen** | EGR-2 |
| **Clone** | REA869 |
| **Isotype** | recombinant human IgG1 |
| **Isotype control** | REA Control antibodies |
| **Alternative names of antigen** | Egr-2, Krox-20, Krox20, Zfp-25 |
| **Entrez Gene ID** | 13654 |
| **Molecular mass of antigen [kDa]** | 50 |
| **Distribution of antigen** | T cells, NK cells |
| **Product format** | Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| **Fixation** | The antibody is suited for staining of formaldehyde-fixed cells. |
| **Storage** | Store protected from light at 2–8 °C. Do not freeze. |

Clone REA869 recognizes the mouse transcription factor EGR-2, also known as early growth response protein 2 or Krox20. EGR-2 consists of three tandem C2H2-type zinc fingers and its expression is induced by EGFR, which is important for osteoprogenitor maintenance and new bone formation. EGR-2 is found primarily in adult thymus, in the embryonic nervous system, and in mammary tumors and plays a critical role in the development of T cells and NKT cells. Additional information: Clone REA869 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® 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 $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.
- FoxP3 Staining Buffer Set (# 130-093-142) for cell fixation and permeabilization to analyze intranuclear proteins or transcription factors by flow cytometry.

## Protocol for intracellular staining of cells

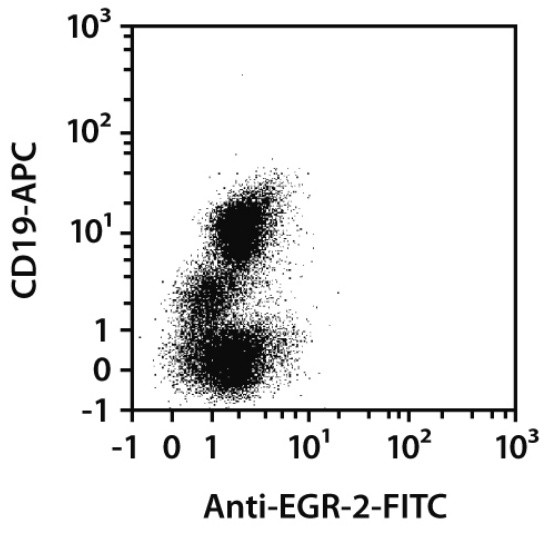
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to $10^6$ cells/100 µL.
- Volumes given below are for up to $10^6$ nucleated cells. When working with fewer than $10^6$ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
- Always prepare Fixation/Permeabilization Solution freshly as described in the data sheet of the FoxP3 Staining Buffer Set (# 130-093-142).

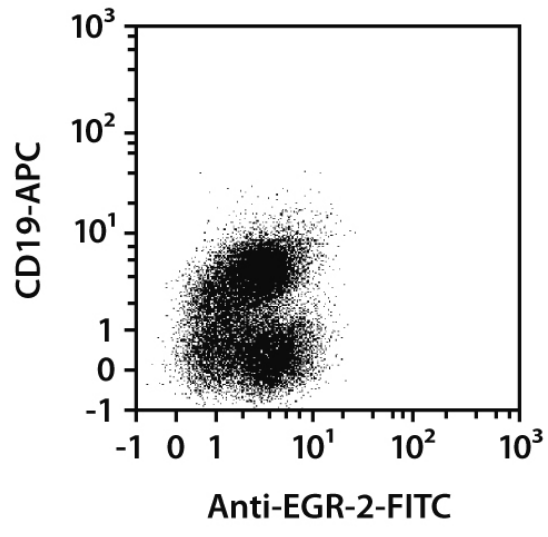
1. Wash up to $10^6$ cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to $10^6$ cells in 1 mL of cold, freshly prepared Fixation/Permeabilization Solution.
4. Mix well and incubate for 30 minutes in the dark in the refrigerator (2−8 °C).
5. Wash cells by adding 1 mL of cold buffer per $10^6$ cells and centrifuge at 300×g for 5 minutes at 4 °C; . Aspirate supernatant completely.
6. Wash cells by adding 1 mL of cold 1× Permeabilization Buffer per $10^6$ cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Resuspend up to $10^6$ nucleated cells in 98 µL of cold 1× Permeabilization Buffer.
8. Add 2 µL of the antibody.
9. Mix well and incubate for 30 minutes in the dark in the refrigerator (2−8 °C).
10. Wash cells by adding 1 mL of cold 1× Permeabilization Buffer per $10^6$ cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

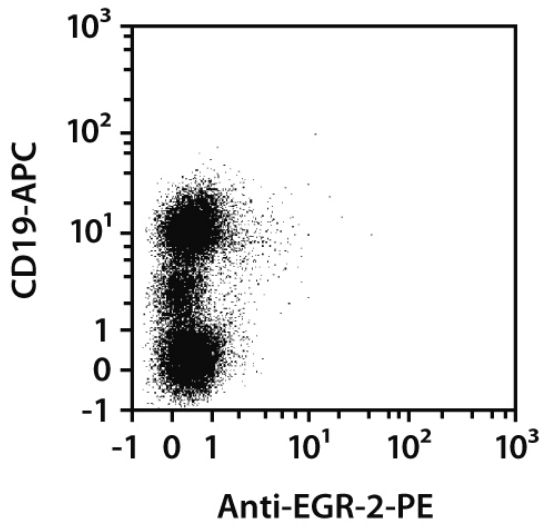
- Note: Do not use propidium iodide (PI) or 7-AAD staining.

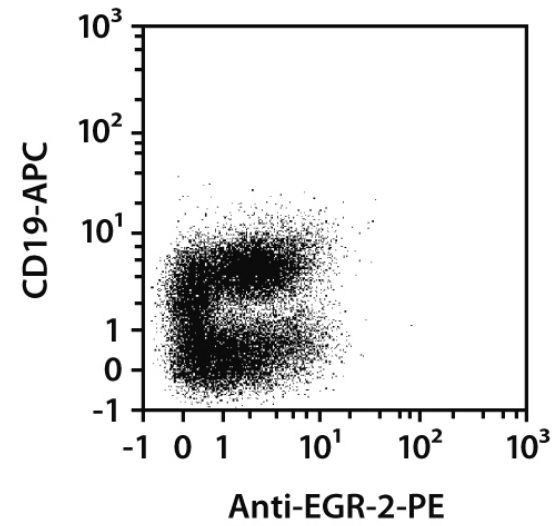
## Examples of immunofluorescent staining

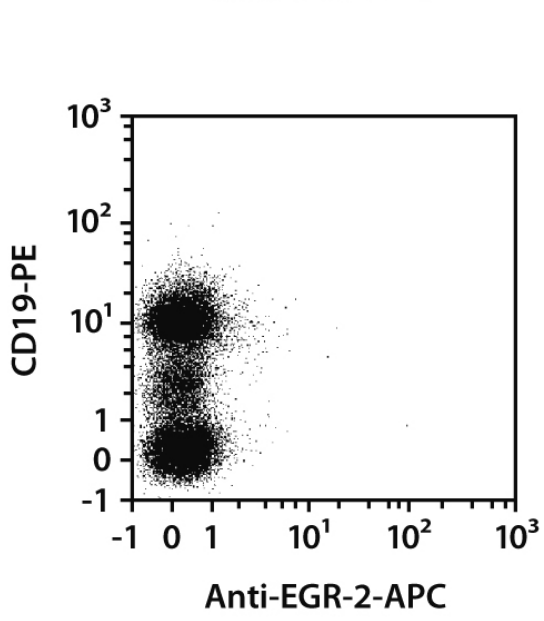
Splenocytes from C57BL/6 mice were either left unstimulated (left image) or stimulated with CD3ε and CD28 antibodies as well as with interleukin 2 (IL-2) over night. Afterwards, PMA and ionomycin were added for two hours. Cells were then fixed and permeabilized using the FoxP3 Staing Buffer Set and stained with Anti-EGR-2 antibodies as well as with CD19 antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris were excluded from the analysis based on scatter signals.

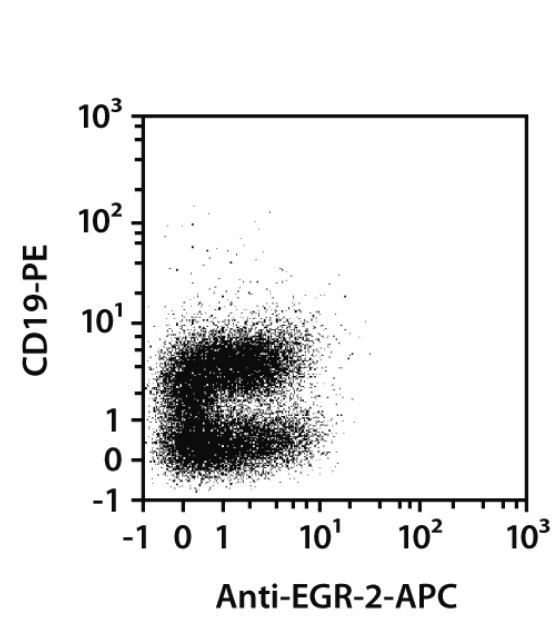


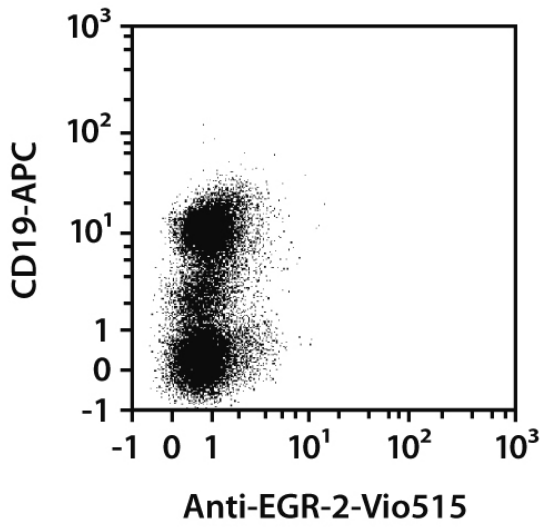



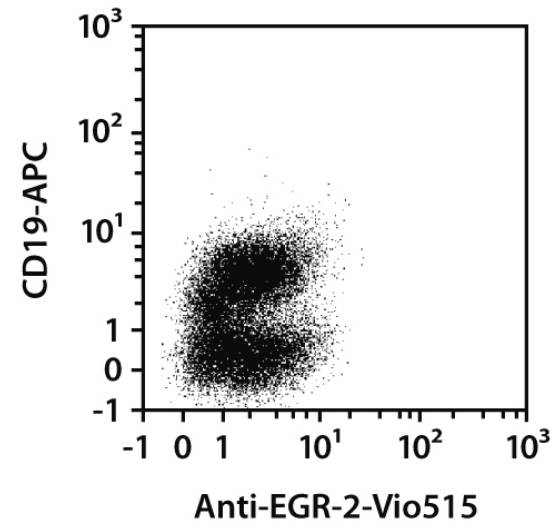
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