CD8b antibodies, human

For research use only

One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
CD8b-FITC	for 100 tests	130-110-509
CD8b-FITC	for 30 tests	130-110-567
CD8b-PE	for 30 tests	130-110-568
CD8b-PE	for 100 tests	130-110-510
CD8b-APC	for 30 tests	130-110-569
CD8b-APC	for 100 tests	130-110-511
CD8b-VioBlue	for 30 tests	130-110-573
CD8b-VioBlue	for 100 tests	130-110-515
CD8b-VioGreen	for 30 tests	130-110-574
CD8b-VioGreen	for 100 tests	130-110-516
CD8b-PE-Vio770	for 30 tests	130-110-570
CD8b-PE-Vio770	for 100 tests	130-110-512
CD8b-APC-Vio770	for 30 tests	130-110-571
CD8b-APC-Vio770	for 100 tests	130-110-513
CD8b-PerCP-Vio700	for 30 tests	130-110-572
CD8b-PerCP-Vio700	for 100 tests	130-110-514
CD8b-Biotin	for 30 tests	130-110-566
CD8b-Biotin	for 100 tests	130-110-508

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	CD8b
Clone	REA715
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CD8 beta chain, CD8 b chain, CD8b
Entrez Gene ID	926
Molecular mass of antigen [kDa]	21

Distribution of antigen	T cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA715 recognizes the human CD8b antigen, a single-pass type I membrane protein and member of the immunoglobulin superfamily, also known as T cell surface glycoprotein Lyt-3. The CD8 glycoprotein is expressed by thymocytes, mature T cells, and natural killer (NK) cells and has been implicated in the recognition of monomorphic determinants on major histocompatibility complex class I antigens and in signal transduction during the course of T cell activation. Both human and rodent CD8 antigens are comprised of two distinct polypeptide chains, alpha and beta. Most of peripheral blood $CD8^+$ T lymphocytes express the CD8a/CD8b heterodimer, while $CD8^+CD16^+$ natural killer cells and $CD8^+$ T cell receptor γ/δ $^+$ T lymphocytes express only the CD8a/CD8a homodimer. CD8b can therefore be used to selectively bind to $CD8^+$ T cells while excluding $CD8^+$ NK cells. Additional information: Clone REA715 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.
- (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.

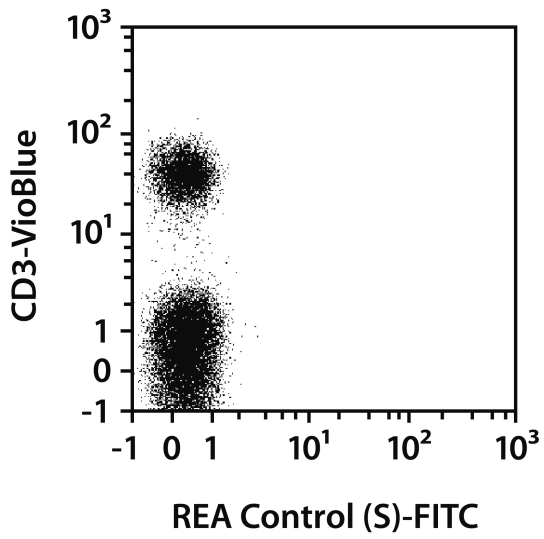
Protocol for cell surface staining

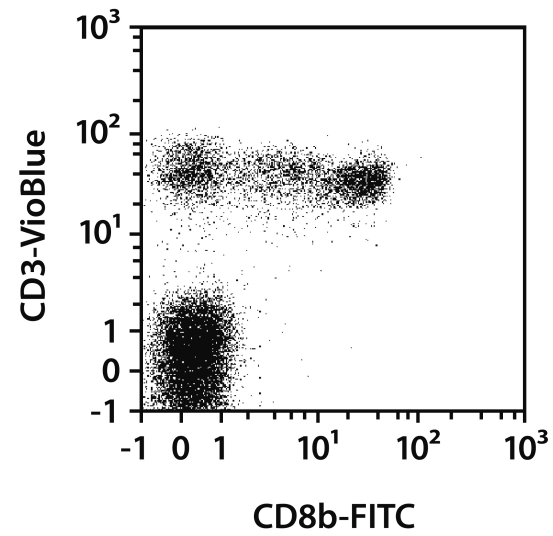
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

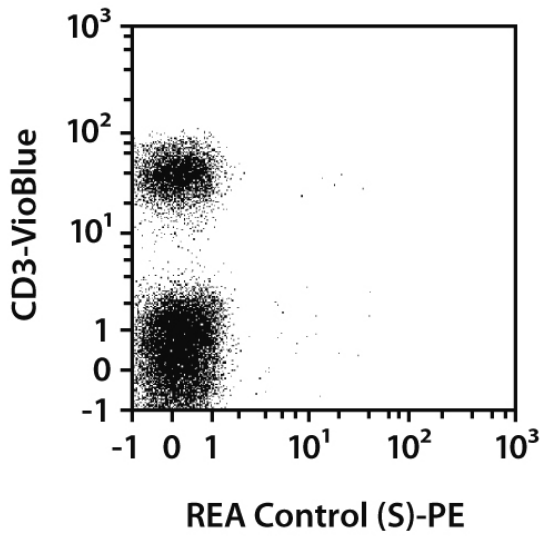
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

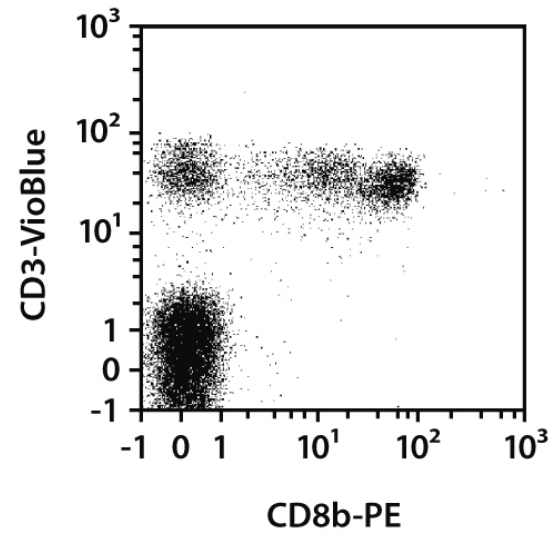
Examples of immunofluorescent staining

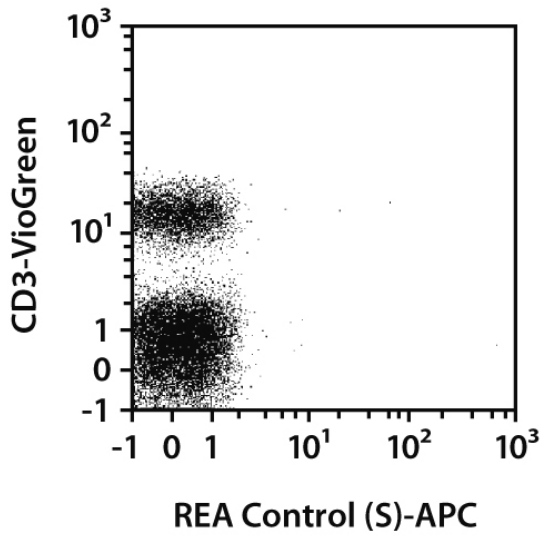
Human peripheral blood mononuclear cells (PBMCs) were stained with CD8b antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD3 antibodies. $CD4^-$ cells were pregated for the analysis. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

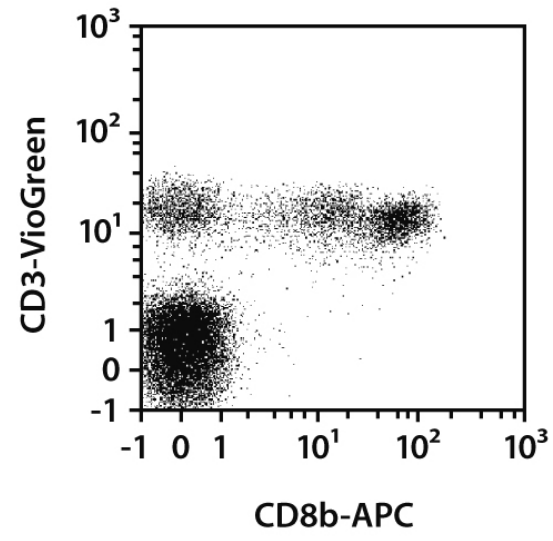

CD3-VioBlue
REA Control (S)-FITC


CD3-VioBlue
CD8b-FITC


CD3-VioBlue
REA Control (S)-PE


CD3-VioBlue
CD8b-PE


CD3-VioGreen
REA Control (S)-APC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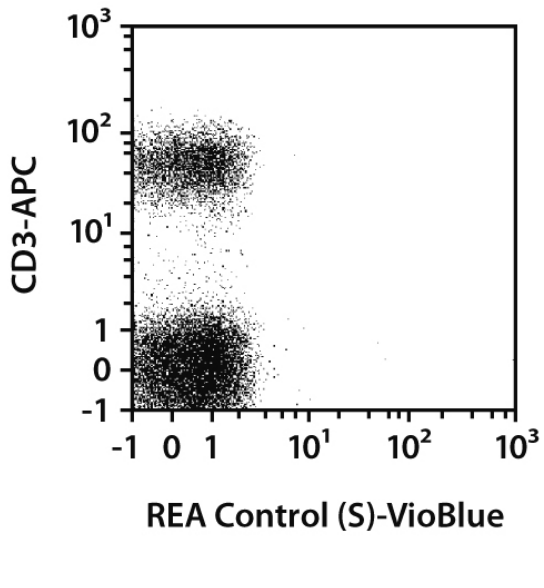
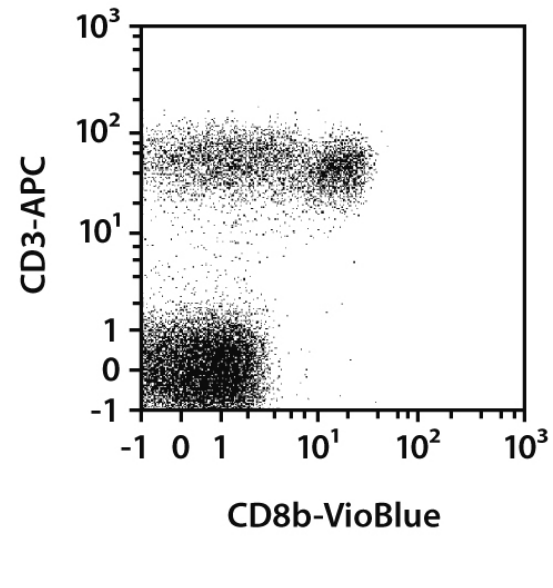
CD8b-APC


CD3-APC
REA Control (S)-VioBlue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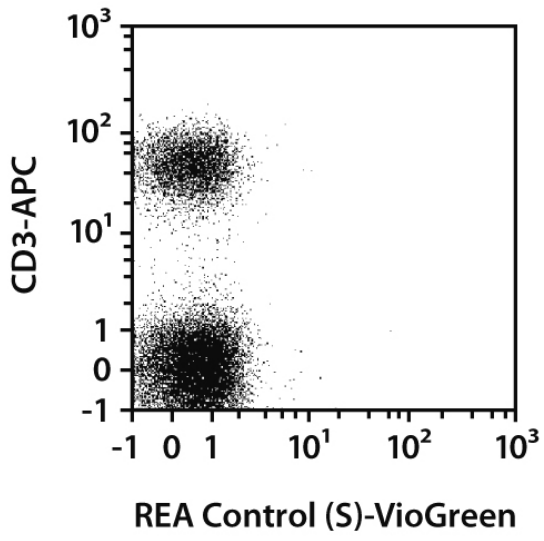
CD8b-VioBlue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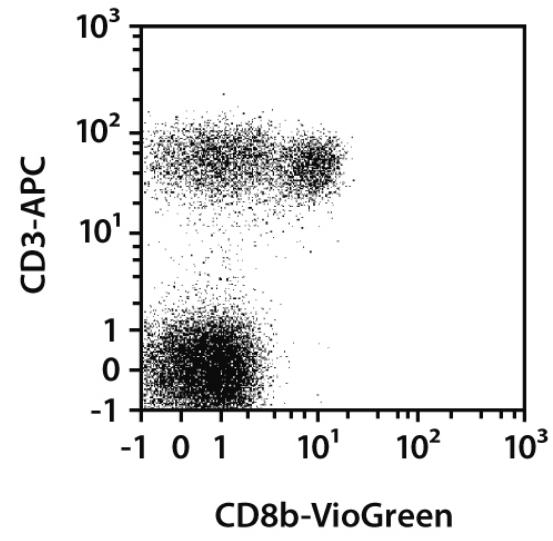
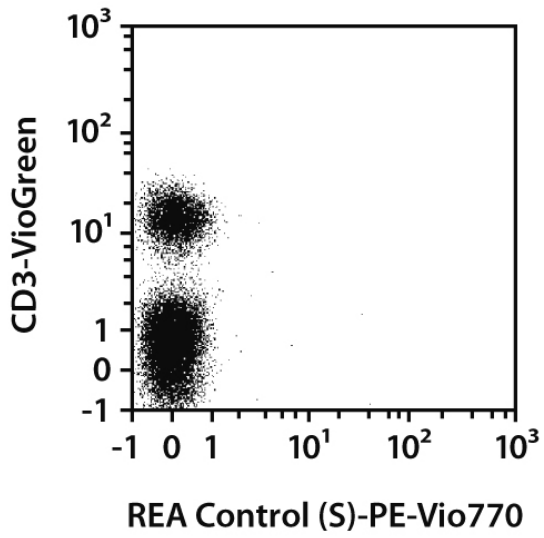
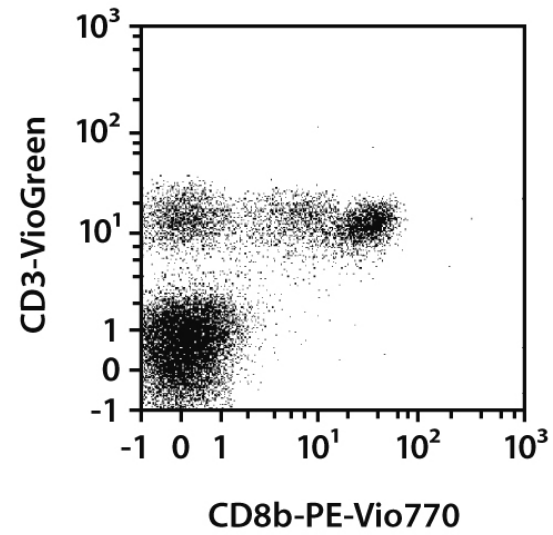
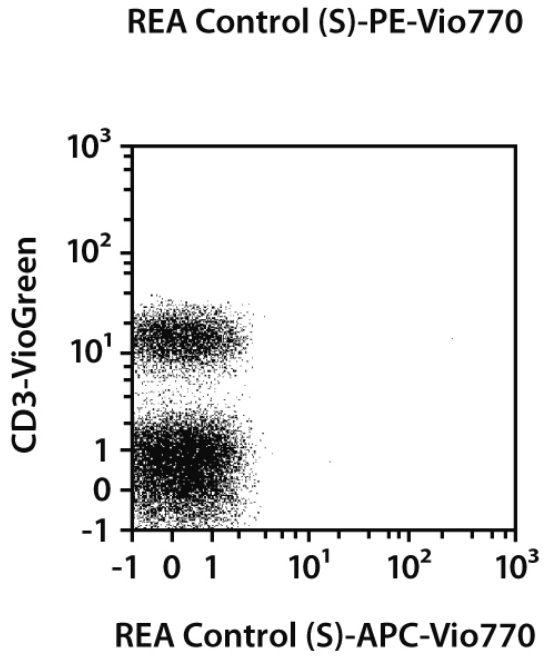
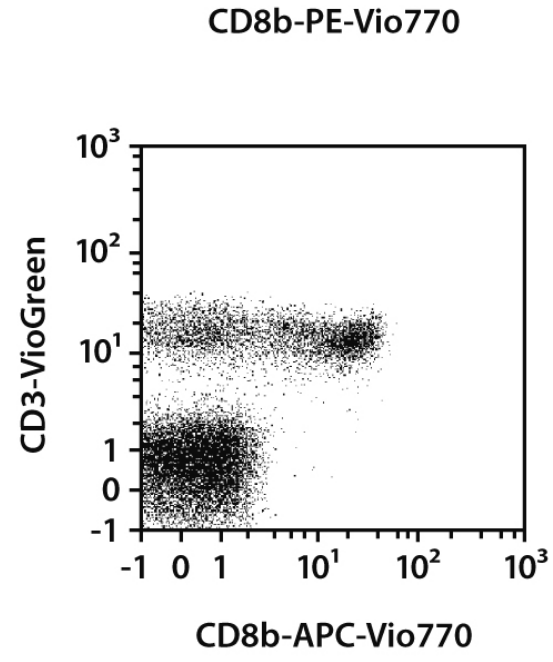
REA Control (S)-VioGreen

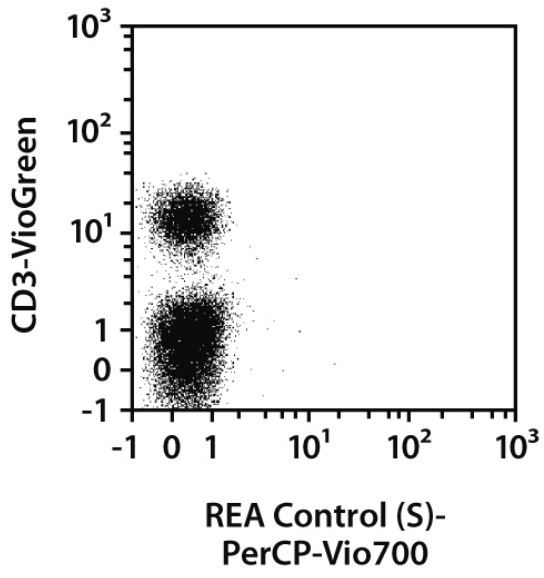

CD3-APC
CD8b-VioGreen

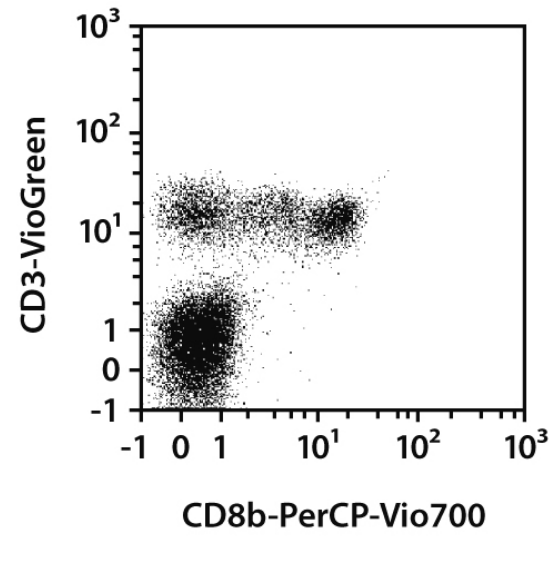


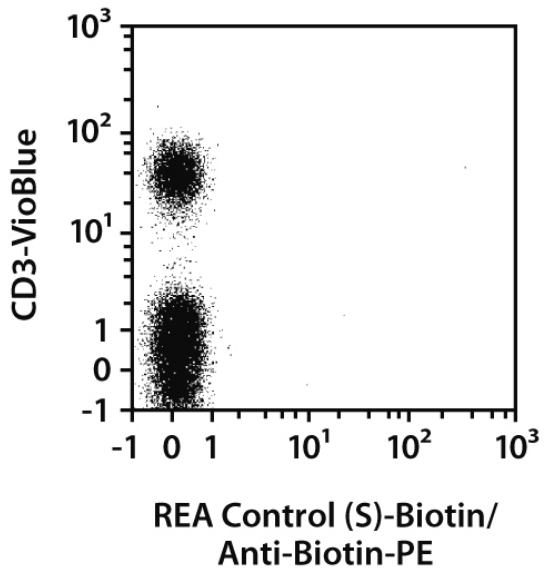


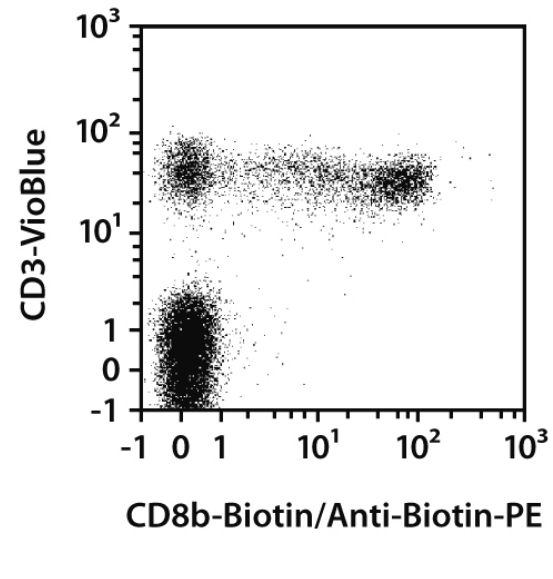












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