Anti-PKCα pT497 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.
Anti-PKCα pT497-FITC	for 100 tests	130-112-473
Anti-PKCα pT497-FITC	for 30 tests	130-112-545
Anti-PKCα pT497-PE	for 30 tests	130-112-546
Anti-PKCα pT497-PE	for 100 tests	130-112-474
Anti-PKCα pT497-APC	for 30 tests	130-112-547
Anti-PKCα pT497-APC	for 100 tests	130-112-475

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	PKCα pT497
Clone	REA821
Isotype	recombinant human IgG1
Isotype control	REA Control (I) antibodies
Alternative names of antigen	PRKCA, AAG6, PKC-alpha, PRKACA, PKC-A
Entrez Gene ID	5578
Molecular mass of antigen [kDa]	77
Distribution of antigen	cancer cells, breast cancer 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 REA821 recognizes the PKCα antigen phosphorylated at threonine 497. PKCα has been reported to crossreact with PKCβ. PKCα is a serine- and threonine-specific protein kinase that can be activated by calcium and the second messenger diacylglycerol. It plays roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control. PKCα is highly expressed in a number of cancer cells where it can acts as a tumor promoter and it is implicated in malignant phenotypes of several tumors such as gliomas and breast cancers. Knockout studies in mice suggest that this kinase may be a fundamental regulator of cardiac contractility and Ca^{2+} handling in myocytes. Additional information: Clone REA821 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.
- Cell Signaling Buffer Set A (# 130-100-827) for cell fixation and permeabilization to analyze intracellular proteins belonging to cell signal transduction pathways or transcription factors by flow cytometry.

Protocol for intracellular staining of cells

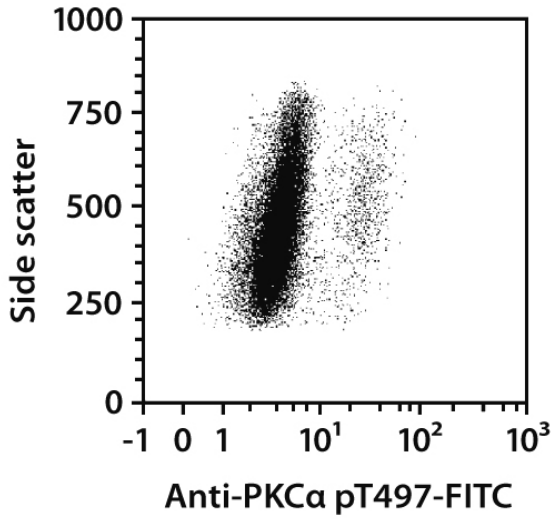
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
- To achieve the appropriate performance for permeabilization of cells, the Permeabilization Buffer A must chill to –20 °C prior to use.

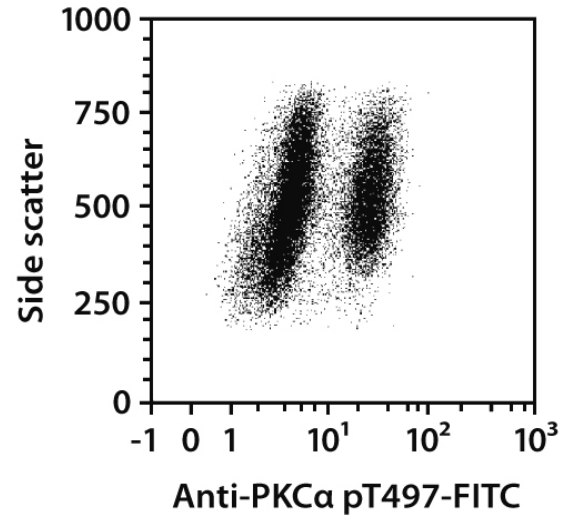
1. (Optional) Treat cells with appropriate cell activators or stimuli.
2. Fix cells by adding 250 µL Inside Fix to 1 mL cell suspension with 10^6 cells.
3. Mix well and incubate for 10 minutes at room temperature.
4. Centrifuge at 500×g for 5 minutes at 4 °C. Aspirate supernatant carefully.
5. Resuspend cells and permeabilize the cells by slowly adding 1 mL of ice-cold (–20 °C) Permeabilization Buffer A per 10^6 cells.
6. Vortex and place the tubes on ice for 30 minutes.
7. Wash cells by adding 3 mL of buffer. Centrifuge at 500×g for 5 minutes at 4 °C. Aspirate supernatant carefully.
8. Wash cells by adding 4 mL of buffer. Centrifuge at 500×g for 5 minutes at 4 °C. Aspirate supernatant carefully.
9. Resuspend up to 10^6 nucleated cells per 98 µL buffer. Add 2 µL of the antibody.
10. Mix well and incubate for 30 minutes in the dark at room temperature.
11. Wash cells by adding 1 mL of buffer and centrifuge at 500×g for 5 minutes. Aspirate supernatant carefully.
12. (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.
13. 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.

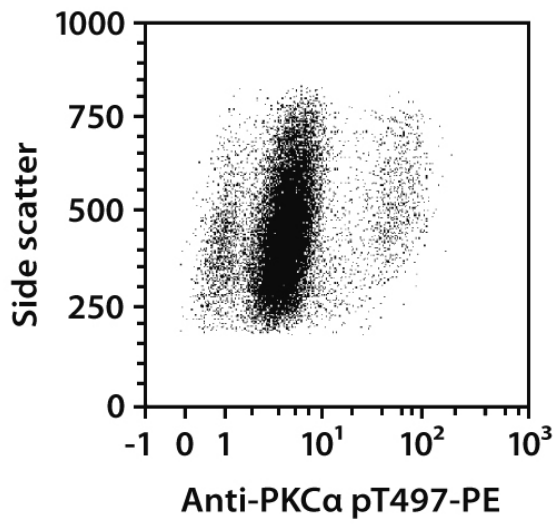
- Note: Do not use propidium iodide (PI) or 7-AAD staining.

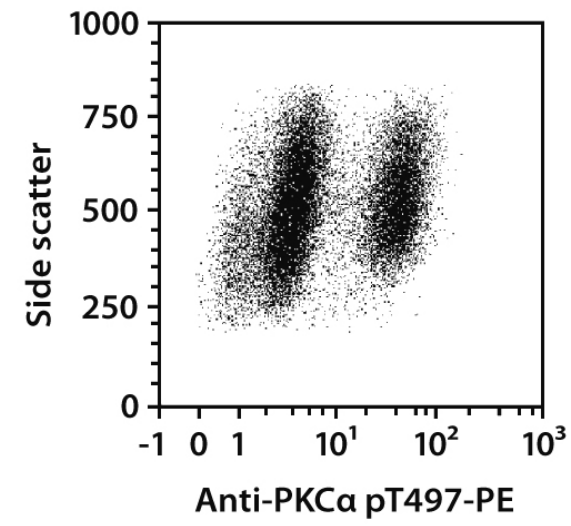
Examples of immunofluorescent staining

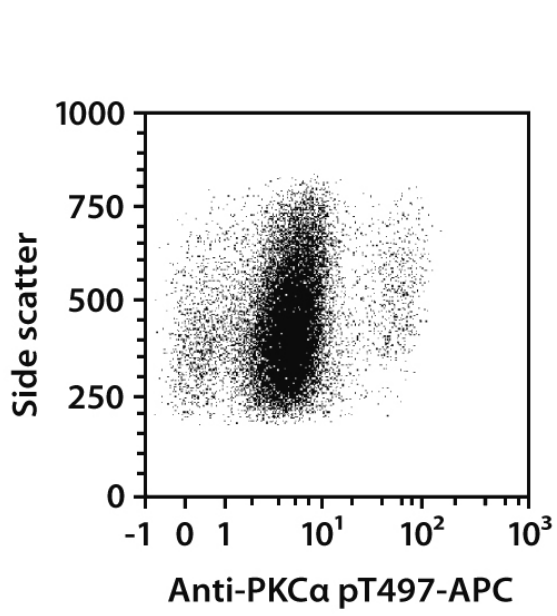
HeLa cells, either left unstimulated (left images) or stimulated with 5 µM nocodazole for 16 hours, were fixed and permeabilized using the Cell Signaling Buffer Set A. Cells were then stained with Anti-PKCα pT497 antibodies and analyzed by flow cytometry using the MACSQuant®Analyzer. Cell debris were excluded from the analysis based on scatter signals.

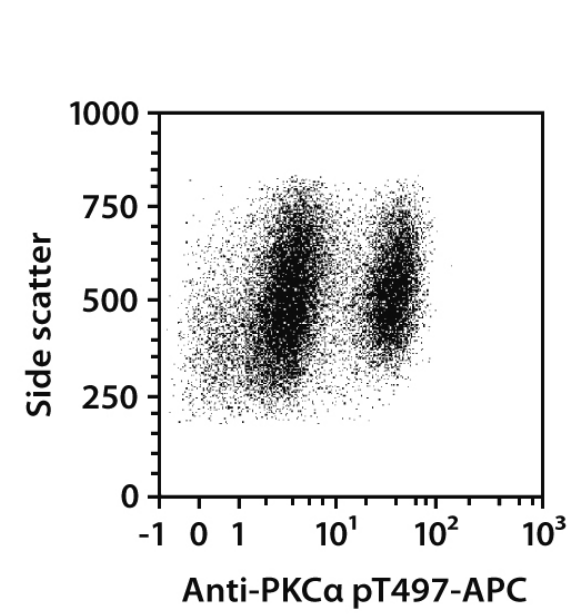












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