



Affinity-Purified Rabbit Anti-human/mouse Caspase 3 Active

ORDERING INFORMATION

Catalog Number: AF835

Lot Number: CFZ33

Size: 50 µg

Storage: -20° C

Specificity: human Caspase 3

Immunogen: human Caspase 3,
aa 163 - 175

Ig Type: Caspase 3 specific rabbit IgG

Applications: Immunohistochemistry

Preparation

Rabbits were immunized with the KLH coupled synthetic peptide CRGTELDCGIETD corresponding to amino acids 163 - 175 of human Caspase 3. Polyclonal antibody was affinity purified on a column derivatized with the peptide.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in 100 µL of PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

On western blots of *E. coli* expressed recombinant Caspase 3, the antibody detects the p17 subunit of Caspase 3 but does not detect or poorly detects the precursor form. This testing was performed on crude extracts containing the recombinant Caspase 3. This antibody can distinguish apoptotic cells from non-apoptotic cells as determined by immunohistochemistry and western blot analysis of anti-Fas treated Jurkat cells and western blots of staurosporine treated mouse 3T3 cells (8 hours, 1 µM). On western blots, cellular extracts from apoptotic cells display multiple bands suggesting that this antibody also detects other apoptotic products. We recommend R&D Systems' Affinity Purified Goat Anti-Caspase 3 (CPP32) (Catalog # AF-605-NA) for western blot applications.

Immunohistochemistry

An antibody concentration of 0.1 - 0.5 µg/mL for staining cultured cells for 30 minutes with antibody is recommended.

For immunohistochemistry images, please refer to our website at <http://www.rndsystems.com/ihc>

Immunohistochemistry of untreated and anti-Fas treated Jurkat cells: Cells were stained with 0.3 µg/mL of rabbit anti-Caspase 3 active for 30 minutes and then Cy3-conjugated anti-rabbit IgG. Cells were counterstained with Fluoro Nissle Green. Quantitation of five fields each of untreated and anti-Fas treated cells indicate that 2.4% of untreated cells and 35% of the anti-Fas treated cells stained positive with anti-Caspase 3 active. The treatment with anti-Fas normally results in approximately 40% of the cells staining positive with Annexin V.

Protocols for Staining Cells with Rabbit Anti-Caspase 3 Active for Immunohistochemistry

Transfer 20 µL of cells (1×10^5 - 1×10^7 cells/mL) in culture medium to an area on a glass slide that is encircled with a PAP pen (Vector Laboratories). For cells grown in suspension, a slide coated with an adhesion matrix is required (e.g. poly-L-lysine). After incubating the slide in a humidified chamber at room temperature for 30 minutes, add an equal volume of freshly made 4% paraformaldehyde in phosphate-buffered saline (PBS) and fix for 20 minutes. Rinse cells 3 times with PBS, 5 minutes per wash. Incubate fixed cells with 0.1 - 0.3 µg/mL of rabbit anti-Caspase 3 active in diluent of choice (e.g. PBS containing 1% bovine serum albumin, 1% normal donkey serum, 0.3% Triton X-100, and 0.01% sodium azide) in a humidified chamber for 30 minutes. Rinse 3 times with PBS, 5 minutes per rinse. Incubate with labeled secondary anti-rabbit IgG for 30 minutes in a humidified chamber. If the secondary antibody is conjugated to a fluorophore, mount a coverslip over the stained cells using an appropriate mounting medium.

When employing HRP-conjugated-avidin for detecting biotinylated secondary reagents, an ABC elite kit (Vector Laboratories) may be used. After incubation for 30 minutes at room temperature, rinse cells 3 times with PBS, 5 minutes per rinse, and add chromogen solution (AEC, DAB, etc.). Counterstain cells (e.g. haematoxylin) and coverslip using either aqueous or non-aqueous mounting medium depending on the chromogen substrate used.

Important suggestions: Use freshly made 4% paraformaldehyde for fixation. When employing HRP as the signal generator, background observed in some cell types may be decreased by use of an avidin-biotin blocking kit (Vector Laboratories) before application of anti-caspase 3 active. When quenching endogenous peroxidase activity, perform the quenching after incubation with anti-Caspase 3 active to ensure maximal antibody-binding. Red blood cells contain a peroxidase-like activity and will stain with HRP substrates in the absence of any added antibodies.

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