

Certificate of Analysis

Anti-ACTIVE® Caspase-3 pAb:

Part No.	Size
G748A	50µl

Description: Anti-ACTIVE® Caspase-3 pAb is intended for use as a marker of apoptosis; it specifically stains apoptotic human cells without staining nonapoptotic cells. Cysteine proteases called caspases are among the mediators of the execution phase of apoptosis. Caspase-3 is a central player in mediating apoptosis and is the most widely studied caspase. All known caspases are synthesized as proenzymes activated by proteolytic cleavage. Anti-ACTIVE® Caspase-3 pAb is an affinity-purified rabbit polyclonal antibody directed against a peptide from the p17 fragment of human caspase-3. The antibody is affinity purified using a peptide corresponding to the cleaved region of caspase-3.

Immunogen: Peptide derived from the cleaved region of caspase-3.

Storage Buffer: Anti-ACTIVE® Caspase-3 pAb is supplied in 1X PBS.

Storage Conditions: For long-term storage, store the undiluted antibody at -20°C. For daily or weekly use, store in aliquots at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See the expiration date on the Product Information Label.

Suggested Working Dilution: 1:250 for immunocytochemistry.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assay

Immunocytochemistry: Anti-ACTIVE® Caspase-3 pAb is tested for its ability to specifically recognize the cleaved active form of caspase-3. Anti-ACTIVE® Caspase-3 pAb selectively stains Jurkat cells that have been treated with Anti-Fas mAb to induce apoptosis and formalin fixed. There is minimal staining of untreated formalin-fixed Jurkat cells with the exception of an occasional dead cell in the untreated population. The antibody bound to active caspase-3 is detected using a secondary anti-rabbit conjugate and a suitable detection system.

Part# 9PIG748

Revised 7/13



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Signed by:

J. Stevens, Quality Assurance

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I. Protocol for Anti-ACTIVE® Caspase-3 pAb Using Cy®3-Conjugated Secondary Antibody

This protocol is a standard immunocytochemical assay demonstrating the use of Anti-ACTIVE® Caspase-3 pAb as a marker for apoptosis in a model system. In this example protocol, Jurkat cells are treated with anti-Fas monoclonal antibody to induce apoptosis. Anti-ACTIVE® Caspase-3 pAb labels anti-Fas mAb-treated Jurkat cells with minimal background staining of untreated Jurkat cells.

A. Anti-Fas mAb Induction of Apoptosis in Jurkat Cells

Grow Jurkat cells in RPMI-1640 medium containing 10% fetal bovine serum in a humidified, 5% CO₂ incubator at 37°C. Suspend the cells in fresh medium to 1×10^5 cells/ml. After two to three days of incubation in a 37°C, 5% CO₂ incubator, harvest the cells by centrifugation at $300\text{--}350 \times g$ for 5 minutes. Resuspend cells in fresh medium to 5×10^5 cells/ml and add anti-Fas mAb to a final concentration of 0.05–0.1 µg/ml. Incubate for 3–6 hours in a 37°C incubator. As a negative control, incubate untreated cells (no anti-Fas mAb) under the same conditions. Harvest the cells by centrifugation at $300\text{--}350 \times g$ for 5 minutes. Remove all medium and resuspend cells in PBS. Repeat centrifugation and resuspend the cell pellet in PBS to 1.5×10^6 cells/ml.

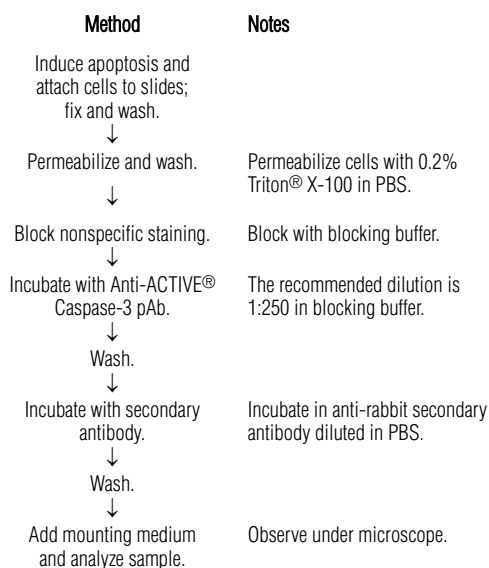
B. Attaching of Cells to Slide, Fixing and Washing

Add a thin layer of anti-Fas mAb-treated and untreated cells to HTC 8-well frosted slides (Fisher Cat.#10-154) that have been previously coated with poly-L-lysine (Sigma Cat.# P9820, diluted 1:10). Do not allow the cells to dry before fixing. The cells will rapidly adhere to the poly-L-lysine. Fix the cells on the slides using 10% neutral buffered formalin in Coplin jars for 25 minutes at room temperature in a fume hood. Wash the slides in 1X PBS for 5 minutes at room temperature. Repeat. Transfer the slides to fresh PBS and store at 4°C (upright). The prepared slides can be stored for several weeks.

C. Detailed Immunochemical Method

1. Permeabilize the fixed cells by incubating in PBS/0.2% Triton® for 5 minutes at room temperature. Wash three times in PBS, in Coplin jars, for 5 minutes at room temperature.
2. Drain the slide and add 200 µl of blocking buffer (PBS/0.1% Tween® 20 + 5% horse serum). Use of serum from the host species of the conjugate antibody (or closely related species) is suggested. Lay the slides flat in a humidified chamber and incubate for 2 hours at room temperature. Rinse once in PBS.
3. Add 100 µl of the Anti-ACTIVE® Caspase-3 pAb diluted 1:250 in blocking buffer. Prepare a slide with no Anti-ACTIVE® Caspase-3 pAb as a negative control. Incubate slides in a humidified chamber overnight at 4°C.
4. The following day, wash the slides twice for 10 minutes in PBS, twice for 10 minutes in PBS/0.1% Tween® 20 and twice for 10 minutes in PBS at room temperature.
5. Drain slides and add 100 µl of donkey anti-rabbit Cy®3 conjugate diluted 1:500 in PBS (we recommend Jackson Laboratories Cat.# 711-165-152). Lay the slides flat in a humidified chamber, **protected from light**, and incubate for 2 hours at room temperature. Wash twice in PBS for 5 minutes, once in PBS/0.1% Tween® 20 for 5 minutes and once in PBS for 5 minutes, protected from light.
6. Drain the liquid, mount the slides in a permanent or aqueous mounting medium and observe under a fluorescence microscope.

D. Flow Chart of General Method



Note: Do not allow the slides to dry during the immunocytochemistry procedure.

II. Related Products

Product	Size	Cat.#
Caspase Inhibitor Z-VAD-FMK	50 µl	G7231
Caspase Inhibitor Ac-DEVD-CHO	100 µl	G5961
CaspACE™ FITC-VAD-FMK In Situ Marker	50 µl	G7461
Anti-PARP p85 Fragment pAb	50 µl	G7341
Anti-pS473 Akt pAb	40 µl	G7441
DeadEnd™ Fluorometric TUNEL System	60 reactions	G3250
DeadEnd™ Colorimetric TUNEL System	20 reactions	G7360
Caspase-Glo® 3/7 Assay	10 ml	G8091
Caspase-Glo® 8 Assay	10 ml	G8201
Caspase-Glo® 9 Assay	10 ml	G8211
CellTiter-Glo® Luminescent Cell Viability Assay	10 ml	G7570
CellTiter 96® AQueous One Solution Cell Proliferation Assay	1,000 assays	G3580
ApoLive-Glo™ Multiplex Assay	10 ml	G6410
rhTNFα	10 µg	G5241

Most products above available in additional sizes.