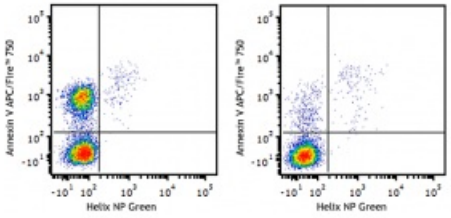
SONY

Product Data Sheet

APC/Fire™ 750 Annexin V

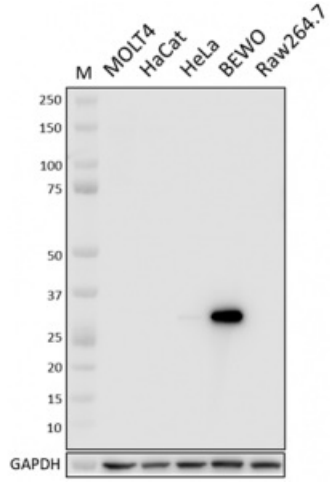
Catalog # / Size:	3804765 / 100 tests 3804760 / 25 tests
Immunogen:	Modified peptide
Reactivity:	Human, Mouse, Other, Rat
Preparation:	The antibody was purified by affinity chromatography and conjugated with APC/Fire™ 750 under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).
Workshop Number:	750 under optimal conditions.
Concentration:	Lot-specific



Human T leukemia cell line Jurkat, treated (left) or non-treated (right) with anti-human CD95 (EOS9.1) mAb (cat. 305704) for 4 hours at 37°C, then stained with Annexin V- APC/Fire™ 750 and Helix NP Green (cat. 425303 at 1.25nM) for 15 minutes at 37°C in Annexin Binding buffer.

Applications:

Applications:	Flow Cytometry
Recommended Usage:	Each lot of this product is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric application, the suggested use of this reagent is 5 - 15 µg per 100,000 - million cells in a 100 µl volume of Annexin V Binding Buffer (Cat No. 422201). It is recommended that the reagent be titrated for optimal performance for each application. * APC/Fire™ 750 has a maximum excitation of 650 nm and a maximum emission of 787 nm.



Whole cell extracts (15 µg protein) from the indicated cell lines were resolved by 4-12% Bis-Tris gel electrophoresis, transferred to a PVDF membrane, and probed with 1.0 µg/mL (1:500 dilution) of purified anti-HMOX1 antibody (clone W19398C) overnight at 4°C. Proteins were visualized by chemiluminescence detection using HRP goat anti-rat IgG antibody (Cat. No. 405405) at 2.0 µg/mL dilution. Direct-Blot™ HRP anti-GAPDH antibody (Cat. No. 607904) was used as a loading control at a 1:50000 dilution (lower). Lane M: Molecular weight marker.

Application Notes:

Annexin V Staining

1. Wash cells twice with cold Staining Buffer and then resuspend cells in Annexin V Binding Buffer at a concentration of $1x10^6$ cells/mL.
2. Transfer 100 µL of cell suspension in 5 mL test tube.
3. Add 5 µL of APC/Fire™ 750 Annexin V.
4. Add 10 µL of PI solution (Cat. No. 421301) or 7-AAD (Cat. No. 420403/420404).
5. Gently vortex the cells, and incubate for 15 min at room temperature (25°C), in the dark.
6. Add 400 µL of Annexin V Binding Buffer to each tube. Analyze by flow cytometry.

Application References:

1. Koopman G, et al. 1994. *Blood* 84:1415.
2. Vermes I, et al. 1995. *J. Immunol. Methods* 184:39.
3. Dachary-Prigent J, et al. 1993. *Blood* 81:2554.
4. Sekine C, *et al.* 2009. *Int Immunol.* PubMed
5. Grujic M, *et al.* 2010. *J. Immunol.* 185:1730. PubMed

Description:

Annexin V (or Annexin A5) is a member of the annexin family of intracellular proteins that binds to phosphatidylserine (PS) in a calcium-dependent manner. PS is normally only found on the intracellular leaflet of the plasma membrane in healthy cells, but during early apoptosis, membrane asymmetry is lost and PS translocates to the external leaflet. Fluorochrome-labeled Annexin V can then be used to specifically target and identify apoptotic cells. Annexin V Binding Buffer (Cat. No. 422201) is recommended for use with Annexin V staining. Annexin V binding alone cannot differentiate between apoptotic and necrotic cells. Therefore, we recommend using our Helix NP™ Blue (Cat. No. 425305), Helix NP™ Green (Cat. No. 425303) or Helix NP™ NIR (Cat. No. 425301). Early apoptotic cells will exclude 7-AAD and PI, while late stage apoptotic cells and necrotic cells will stain positively, due to the passage of these dyes into the nucleus where they bind to DNA.

Antigen References:

1. Yang B, *et al*. 2018. *Toxicol Appl Pharmacol.* 355:189-197
2. Chora A, *et al.* 2007. *J Clin Invest.* 117(2):438-47
3. Harding H, *et al.* 2003. *Mol Cell.* 11(3):619-33.