abcam

Product datasheet

Caspase-8 (active) FITC Staining Kit ab65614

1 References 3 Images

Overview

Product name	Caspase-8 (active) FITC Staining Kit
Sample type	Adherent cells, Suspension cells
Assay type	Enzyme activity
Assay time	2h 00m
Product overview	Caspase 8 (active) FITC Staining Kit (ab65614) provides a convenient means for sensitive detection of activated caspase 8 in living cells. The assay utilizes the caspase 8 inhibitor, IETD-FMK, conjugated to FITC (FITC-IETD-FMK) as a marker. FITC-IETD-FMK is cell permeable, non-toxic, and irreversibly binds to activated caspase 8 in apoptotic cells. The FITC label allows detection of activated caspase-8 in apoptotic cells directly by fluorescence microscopy, flow cytometry, or fluorescence plate reader. Visit our FAQs page for tips and troubleshooting.
Notes	Activation of caspases plays a central role in apoptosis. **Other caspase and apoptosis assays** Review the full set of caspase assays, or the apoptosis assay and apoptosis marker guide.
Platform	Microplate reader, Fluor. microscope, Flow cyt.

Properties

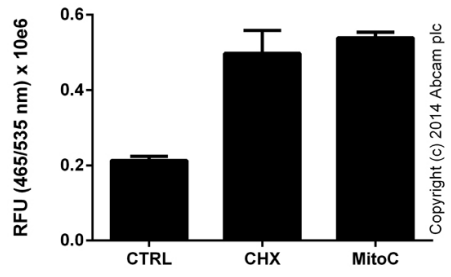
Storage instructions	Store at -20°C. Please refer to protocols.

Components	100 tests
FITC-IETD-FMK	1 x 100µl
Wash Buffer	2 x 100ml
Z-VAD-FMK	1 x 10µl

Function	Most upstream protease of the activation cascade of caspases responsible for the TNFRSF6/FAS mediated and TNFRSF1A induced cell death. Binding to the adapter molecule FADD recruits it to either receptor. The resulting aggregate called death-inducing signaling complex (DISC) performs CASP8 proteolytic activation. The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases. Proteolytic fragments of the N-terminal propeptide (termed CAP3, CAP5 and CAP6) are likely retained in the

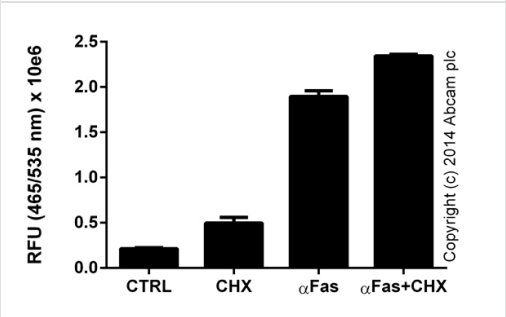
	DISC. Cleaves and activates CASP3, CASP4, CASP6, CASP7, CASP9 and CASP10. May participate in the GZMB apoptotic pathways. Cleaves ADPRT. Hydrolyzes the small-molecule substrate, Ac-Asp-Glu-Val-Asp- -AMC. Likely target for the cowpox virus CRMA death inhibitory protein. Isoform 5, isoform 6, isoform 7 and isoform 8 lack the catalytic site and may interfere with the pro-apoptotic activity of the complex.
Tissue specificity	Isoform 1, isoform 5 and isoform 7 are expressed in a wide variety of tissues. Highest expression in peripheral blood leukocytes, spleen, thymus and liver. Barely detectable in brain, testis and skeletal muscle.
Involvement in disease	Defects in CASP8 are the cause of caspase-8 deficiency (CASP8D) [MIM:607271]. CASP8D is a disorder resembling autoimmune lymphoproliferative syndrome (ALPS). It is characterized by lymphadenopathy, splenomegaly, and defective CD95-induced apoptosis of peripheral blood lymphocytes (PBLs). It leads to defects in activation of T-lymphocytes, B-lymphocytes, and natural killer cells leading to immunodeficiency characterized by recurrent sinopulmonary and herpes simplex virus infections and poor responses to immunization.
Sequence similarities	Belongs to the peptidase C14A family. Contains 2 DED (death effector) domains.
Domain	Isoform 9 contains a N-terminal extension that is required for interaction with the BCAP31 complex.
Post-translational modifications	Generation of the subunits requires association with the death-inducing signaling complex (DISC), whereas additional processing is likely due to the autocatalytic activity of the activated protease. GZMB and CASP10 can be involved in these processing events. Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Cytoplasm.

Images



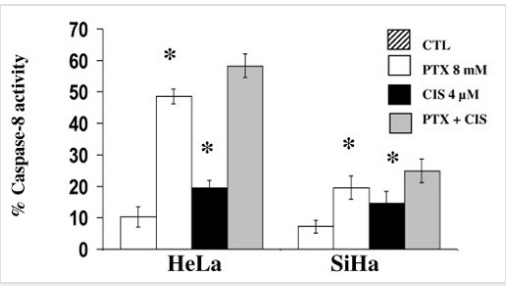
Functional assays: Caspase 8 (active) FITC Staining Kit (ab65614)

Active caspase 8 in control Jurkat cells (10e6/mL) or cells treated for five hours with 10 ug/mL Cyclohexamide (CHX) (ab120093) or four hours with 25 ug/mL Mitomycin C (MitoC) (ab120797). Background signal subtracted, duplicates; +/- SD.



Functional assays: Caspase 8 (active) FITC Staining Kit (ab65614)

Active caspase 8 in Jurkat cells following four hours exposure to 50 ng/mL anti-Fas Ab (αFas) (MBL), five hours with 10 µg/mL cyclohexamide (CHX) (ab120093), or one hour pretreatment with CHX followed by four hours with αFas. Background signal subtracted, duplicates; +/- SD.



Flow Cytometry - Caspase 8 (active) FITC Staining Kit (ab65614)

Caspase 8 activation of Hela (left) and SiHa (right) cells after *in vitro* treatment with pentoxylline (PTX) or cisplatin (CIS) either alone or in combination. Results represent the mean ± SD of three independent experiments carried out in triplicate. (*) $p<0.001$ vs CTL.

Image obtained from Hernandez-Flores G et al; BMC Cancer, 2011 Nov 11; 11:483

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