# abcam

### Product datasheet

## Caspase-8 Assay Kit (Colorimetric) ab39700

38 References 画像数 2

#### 製品の概要

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製品名 Caspase-8 Assay Kit (Colorimetric)

検出方法 Colorimetric サンプルの種類 Cell Lysate アッセイタイプ Enzyme activity

2h 00m

全工程の試験時間

Caspase 8 Assay Kit (colorimetric) (ab39700) is a simple and convenient assay to quantify the activity of caspase 8 in cell lysates, based on the recognition of the sequence lle-Glu-Thr-Asp (IETD). The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after it is cleaved from the labeled substrate IETD-pNA. The pNA light emission can be quantified using a spectrophotometer or a microtiter plate reader at OD 400 – 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with an un-induced control allows determination of the fold increase in Caspase 8 activity.

Visit our **FAQs page** for tips and troubleshooting.

This product is manufactured by BioVision, an Abcam company and was previously called K113 Caspase-8 Colorimetric Assay Kit. K113-100 is the same size as the 100 test size of ab39700.

The caspase family of highly conserved cysteine proteases play an essential role in programmed cell death (including apoptosis, pyropoptosis and necroptosis).

Mammalian caspases can be subdivided into three functional groups: initiator caspases (Caspase 2, 8, 9 and 10), executioner caspases (Caspase 3, 6 and 7), and inflammatory caspases (Caspase 1, 4, 5, 11 and 12). Initially synthesized as inactive pro-caspases, caspases become rapidly cleaved and activated in response to granzyme B, death receptors and apoptosome stimuli. Caspases will then cleave a range of substrates, including downstream caspases, nuclear proteins, plasma membrane proteins and mitochondrial proteins, ultimately leading to cell death.

Caspase 8 (CASP8/FLICE, EC:3.4.22.61) is the most upstream caspase involved in the activation of apoptosis through the extrinsic pathway, mediated by CD95 (Fas) receptor and TNFR. Caspase 8 is recruited to the receptors through the adapter molecule FADD, resulting in the formation of the aggregate called death-inducing signaling complex (DISC) and proteolytic activation of caspase 8. The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases. Inhibition or inactivation of caspase 8 is required for induction of necroptosis.

#### 特記事項

#### Other caspase and apoptosis assays

Review the full set of caspase assays, or the apoptosis assay and apoptosis marker guide.

#### 試験プラットフォーム

Microplate reader

#### 製品の特性

#### 保存方法

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

内容	100 tests
2X Reaction Buffer I	4 x 2ml
Dilution Buffer II	1 x 100ml
DTTI	1 x 400µl
IETD-pNA	1 x 500µl
Lysis Buffer IV	1 x 100ml

#### 機能

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.

#### 組織特異性

lsoform 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.

#### 関連疾患

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.

#### 配列類似性

Belongs to the peptidase C14A family.

Contains 2 DED (death effector) domains.

ドメイン

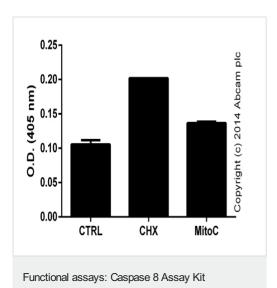
Isoform 9 contains a N-terminal extension that is required for interaction with the BCAP31 complex.

#### 翻訳後修飾

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.

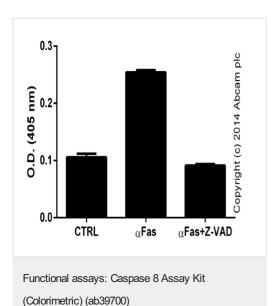
Phosphorylated upon DNA damage, probably by ATM or ATR.

#### 画像



(Colorimetric) (ab39700)

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



Active caspase 8 in control (CTRL) Jurkat cells (10e6/mL) or in cells after four hours exposure to 50 ng/mL anti-Fas Ab ( $\alpha$ Fas) (MBL), or pretreated one hour with 50  $\mu$ M Z-VAD(OMe)-FMK ( $\alpha$ b120487) followed by four hours with  $\alpha$ Fas. Background signal subtracted, duplicates; +/- SD.

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