

Caspases, Proteases

Apoptosis is a selective, controlled, and genetically programmed cell death process, which occurs as a result of normal cellular differentiation and development of multicellular organisms. It is also involved in tissue homeostasis, aging, and pathological processes. Apoptosis is induced by caspases which appear to participate in a cascade of cleavage events that contribute to the disabling of homeostatic and repair processes in addition to the systematic structural disassembly of dying cells. An imbalance in apoptosis appears to underlie the aetiology of many human diseases that may involve either insufficient apoptotic death, such as cancer, or excessive or premature apoptosis, such as Alzheimer's disease. Thus, caspases are a common target to generate drugs for therapeutic intervention in these diseases.

The cysteine protease CPP32 or Caspase 3, a key mediator of apoptosis, has been expressed at IBA in *Escherichia coli* as a 32 kDa proprotein with C-terminal *Strep*-tag II. The proprotein is processed by autoprotoleolysis or by an *E. coli* protease to yield p17 and p12, the latter of which still carries the *Strep*-tag II. The resulting active heterotetramer (p17/p12-*Strep*)₂ is susceptible to further autoprotoleolytic degradation (especially p17; see lane 5). Thus *Strep*-tag is shown to be very suitable to enable the one-step isolation of functional caspase 3. One run on a *Strep*-Tactin column with 1 ml bed volume yielded 0.7 mg caspase 3 in the described experiment (Figure 1).

Figure 1

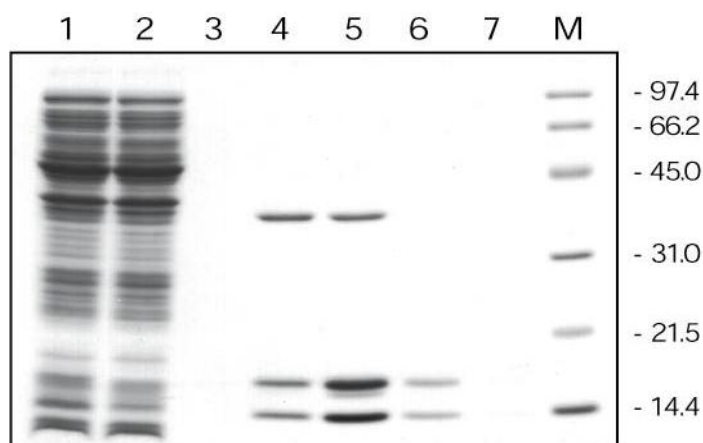


Figure 1: Purification of CPP32 from *E. coli* crude cell extract.

The CPP32 cDNA from the human hepatocyte cell line hUH7 was PCR amplified and inserted into pASK-IBA3 in order to achieve C-terminal *Strep*-tag II fusion. Gene expression was achieved in this case at 25 °C in 200 ml XL1-Blue. After cell harvest, cells were resuspended in 2 ml buffer W. The cytosolic cell extract was prepared by saving the supernatant after sonication and centrifugation. As a modification to the standard procedure 150 mM NaCl and 3 mM DTT were added to buffer W (100 mM Tris-Cl pH 8.0, 150 mM NaCl) during preparation of the lysate and chromatography. 2 ml of the lysate were purified on a column with 1 ml bed volume after washing the column with 5x 1 ml washing buffer and applying 6x 0.5 ml washing buffer including 2.5 mM desthiobiotin. The whole procedure yielded 0.7 mg active caspase 3.

Lane 1: Cytosolic lysate; lane 2: column eluate after the first washing step; lane 3-7: column eluates from the second to sixth elution step; and lane M: molecular size standard (kDa).