New Apoptosis Reagents

Efficient FasL-Assays

2009

Heterogeneous and Homogeneous FasL-Strep Apoptosis Assays

FasL in Apoptosis and Disease

Apoptosis, a form of programmed cell death in multicellular organisms, is directly triggered by FasL(igand)/Fas system. FasL, also known as CD95L, plays a pivotal role in regulating normal B and T cell function, suppression of autoimmunity, control of infection and immune surveillance. As a result of its dual role, namely, in self-control of T cell expansion and in killing of virally infected or neoplastically transformed target cells, Fas/FasL system is involved in disease areas such as autoimmune diseases, cancer and immune deficiency, which are all well-known features of neoplastic diseases, in particular, of the lympho-hematopoietic system.

FasL binds the Fas receptor (also known as Apo-1 or CD95), a trans- membrane protein, which is part of the TNFR superfamily. The interaction between Fas receptor and FasL results in the formation of the death-inducing signaling complex (DISC), which contains FADD, caspase-8 and caspase-10. In many cell types, processed pro-caspase-8 directly activates other members of the caspase family and triggers the execution of apoptosis.

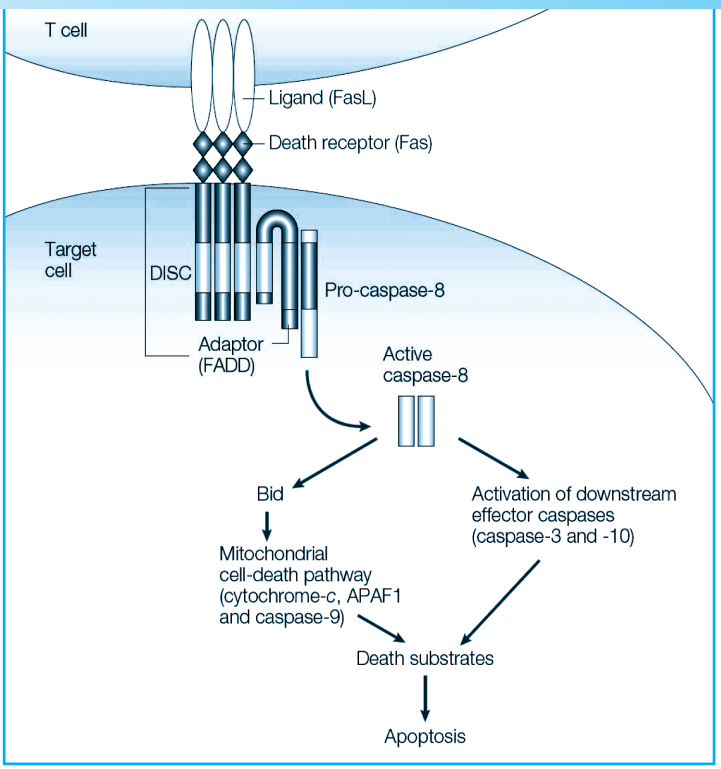

Figure from Nat Rev Immunol 2, 2002, 273-281.

FasL, the T4 Foldon and Strep-tag®...

As type II transmembrane protein FasL is localized in the cell membrane of T-cells, its homotrimerization is a prerequisite for apoptotic activity. Thus, the stabilization of the functional conformation of the trimeric extracellular receptor binding domain (RBD) of FasL is mandatory for its use as apoptotic reagent. A stable trimeric conformation of FasL has been engineered by introduction of the "T4-FOLDON", a small trimeric globular domain derived from the C-terminus of the bacteriophage T4 protein fibritin. In contrast to commonly used N-terminal fused coiled-coil structures, C-terminal fusion of the T4-FOLDON to the FasL-RBD results in superior bioactive, stable, well defined trimers.

The presented data are property of Apogenix GmbH.
Trimerized Fas ligands are object of patent applications by Apogenix GmbH.
For further information see also Kleber et al., 2008, Cancer Cell 13, 235-248.

IBA BioTAGnology

...distributed by:

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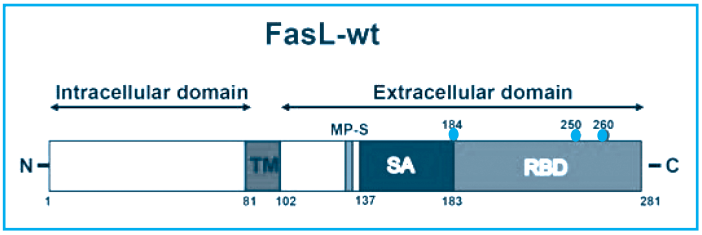
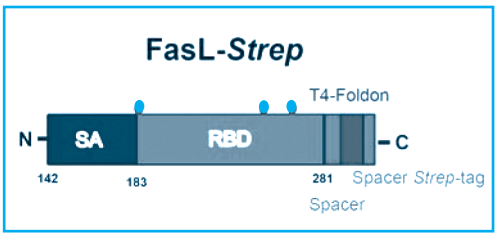
...the winning combination

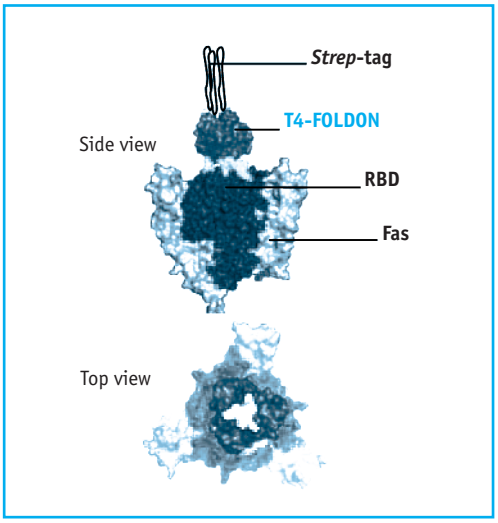
In addition to the T4-FOLDON, the FasL reagent contains a Strep-tag® fused to each of the three monomers. The Strep-tag® allows not only an optimal purification but also immobilization and detection of the FasL reagent. Thus Strep-tag® is ideal for the handling of this novel FasL-reagent named FasL-Strep.



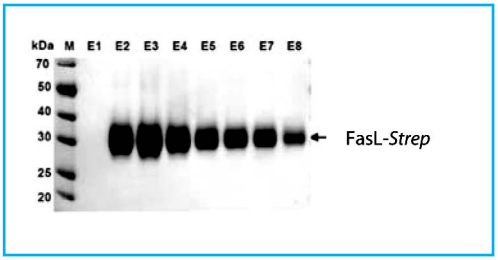


The recombinant FasL-Strep is produced in HEK 293 cells by a secretion strategy enabling authentic glycosylation

TM: Transmembrane domain
MP-S: Metalloprotease substrate sites
SA: Self-assembly and trimerization region
RBD: Receptor binding domain
●: Glycosylation site

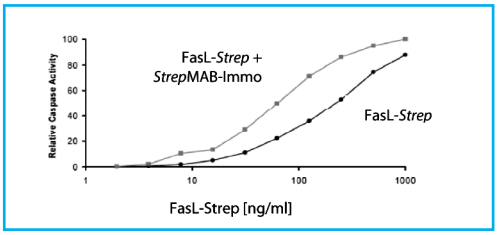


Model based on the structures of the TRAIL/DR5 complex and the T4-FOLDON illustrating the putative structure of the trimeric FasL-Strep/Fas complex

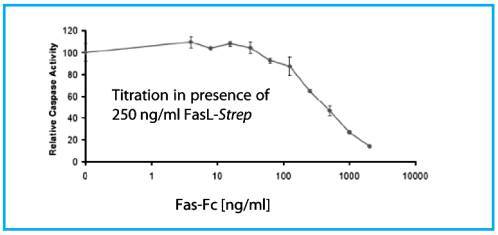


One-Step purification of FasL-Strep on Strep-Tactin Sepharose

SDS-PAGE and silver staining reveal an apparent MW of 30 kDa of the FasL-Strep monomer and a purity of >95%



Apoptotic activity of FasL-Strep is enhanced by cross-linking via the monoclonal anti-body StrepMAB-Immo



Apoptotic activity of FasL-Strep is specifically blocked by Fas-Fc

Heterogeneous FasL-Strep Apoptosis Assay

In the heterogeneous apoptosis assay format, the FasL-Strep molecule is immobilized on Strep-Tactin® (the cognitive receptor of Strep-tag®) coated microplates. After addition of the cells to be investigated, caspase 3/7 activity is determined for measuring the extent of apoptosis. The FasL-Strep apoptosis assay described above is robust and reproducible due to the high stability of the FasL-Strep reagent. The test is easy to perform since common reagents are applied for read out. These properties identify this assay as an ideal basis for HTS assays for screening anti-apoptotic molecules. The assay is available for human or murine FasL on Strep-Tactin® coated microtiter-plates.

Homogeneous FasL-Strep Apoptosis Assay

The homogeneous FasL-Strep Apoptosis Assay can be performed in the presence of the anti-Strep-tag® antibody "StrepMAB-Immo" which increases the apoptotic activity of FasL-Strep. The homogeneous FasL-Strep assay enables the quantitative determination of anti-apoptotic agents as shown by Fas-Fc competition.
The measurement of apoptosis in the respective cells is achieved by determination of caspase 3/7 activity. The assay is available with human or murine FasL-Strep and includes StrepMAB-Immo.

Order Information:

FasL-Strep Apoptosis Assays

Cat.No.	Product	Amount	Price in €
2-3911-000	Heterogeneous FasL-Strep Apoptosis Assay, human*	96 assays	172,50
2-3912-000	Homogeneous FasL-Strep Apoptosis Assay, human*	96 assays	437,00
2-3961-000	Heterogeneous FasL-Strep Apoptosis Assay, murine*	96 assays	149,50
2-3962-000	Homogeneous FasL-Strep Apoptosis Assay, murine*	96 assays	414,00
2-3901-002	Human FasL-Strep*	2.5 µg	109,25
2-3901-010	Human FasL-Strep*	10 µg (4x2.5µg)	339,25
2-3951-002	Mouse FasL-Strep*	2.5 µg	86,25
2-3951-010	Mouse FasL-Strep*	10 µg (4x2.5µg)	287,50
2-1517-001	StrepMAB-Immo; purified	100 µg	287,50
2-1501-001	Strep-Tactin® coated microplate	1 plate	46,00
2-1501-050	Strep-Tactin® coated microplates	5 plates	218,50
BP0012	Ac-DEVD-AFC, CAS Number: 201608-14-2	5 mg	135,00

**Storage -80 °C, extra shipping costs*

All prices are net, plus VAT and freight charges where applicable.