

# FasL-Strep

Novel FasL and FasL-Assays



*Dead or alive?*

**IBA's Apoptosis Reagents Provide Insight**

# FasL in Apoptosis and Disease

Apoptosis, a form of programmed cell death in multicellular organisms, is directly triggered by FasL (ligand)/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 transmembrane 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.

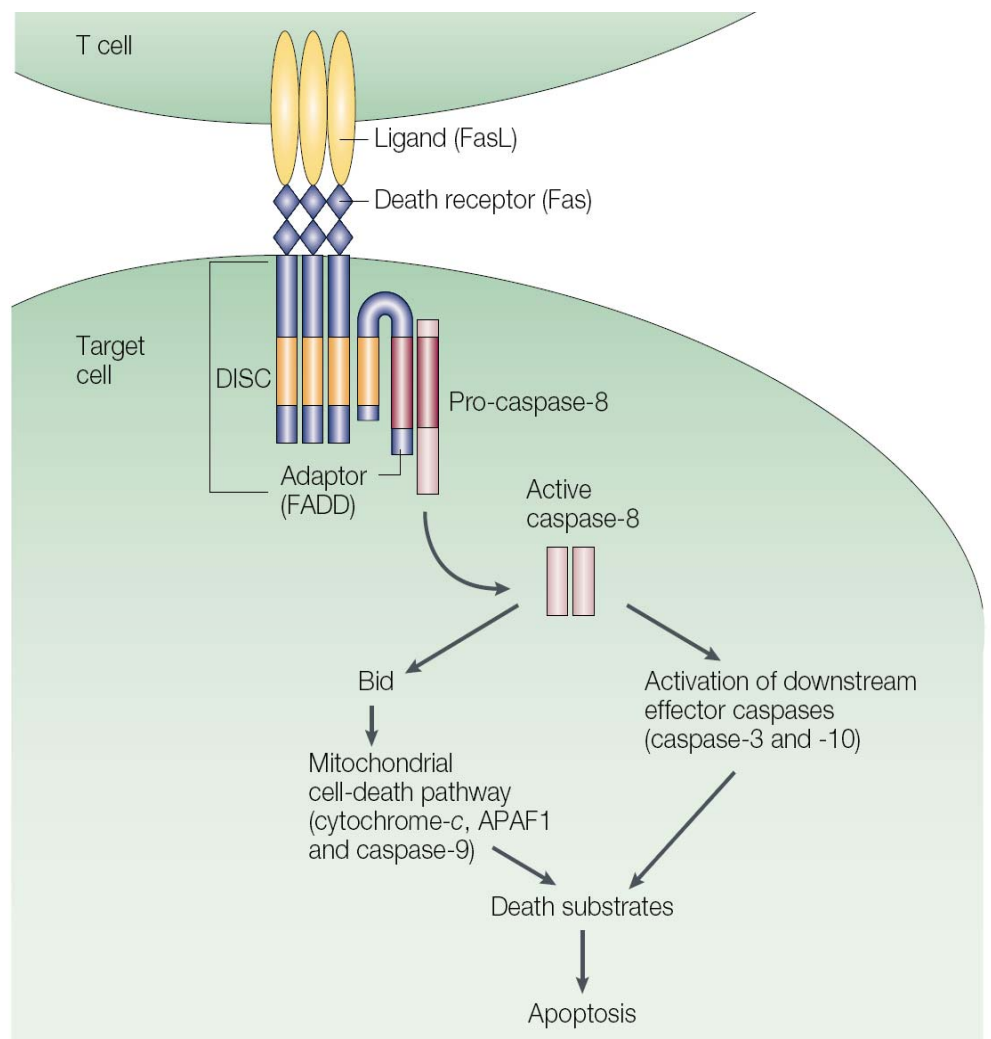


Figure from Nat Rev Immunol 2, 2002, 273-281.

# FasL, the T4 Foldon and *Strep-tag*<sup>®</sup>

## - The winning combination -

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.

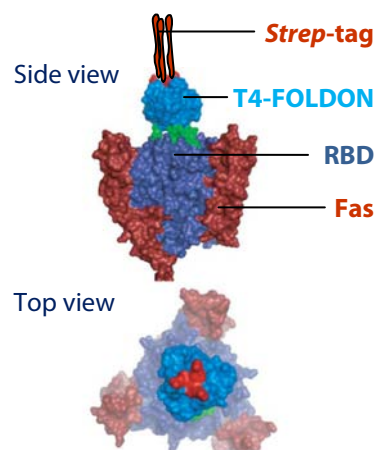
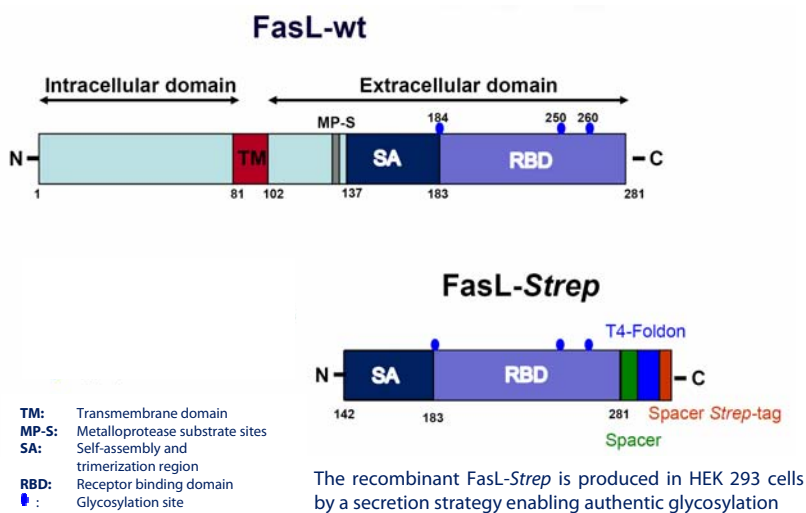
A stable trimeric conformation of FasL has been engineered by introduction of the "T4-FOLDON" a small trimeric globular domain derived from the C-terminus of the bacteriophage T4 protein fibrinin. In contrast to commonly used N-terminal fused coiled-coil structures, C-terminal fusion of the T4-FOLDON to the FasL-RBD results in superior bioactive, stable, well defined trimers.

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

### Advantages of FasL-*Strep*

- Defined trimeric structure
- High stability
- Efficient apoptosis induction
- Easy handling (detection, immobilization, isolation) via *Strep-tag*<sup>®</sup>
- Competitive pricing

## FasL-*Strep* – Molecular structure



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

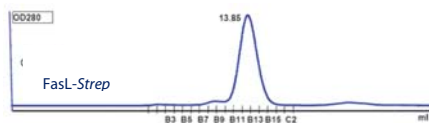
## FasL-*Strep* – A pure and stable trimeric reagent

### 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%

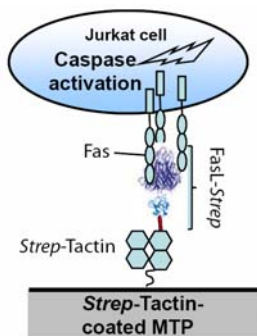
### Molecular weight determination by size exclusion chromatography



FasL-*Strep* is a homogenous and stable trimer. A molecular weight of 90 kDa was determined

# FasL-*Strep* Apoptosis Assays

## Heterogeneous FasL-*Strep* Apoptosis Assay



FasL-*Strep* is immobilized on *Strep*-Tactin® coated microplates and apoptosis is specifically induced in Fas expressing cells

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 extent of apoptosis.

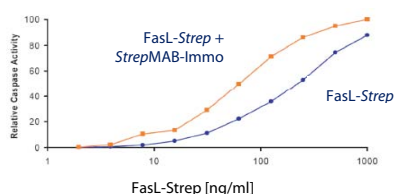
The FasL-*Strep* apoptosis assay described above is robust and reproducible due to the high stability of the FasL-*Strep* reagent. The test is easy to perform since common reagents are applied for read out. These properties identify this assay as an ideal basis for HTS assays for screening anti-apoptotic molecules.

The assay is available for human or murine FasL on *Strep*-Tactin® coated MTP.

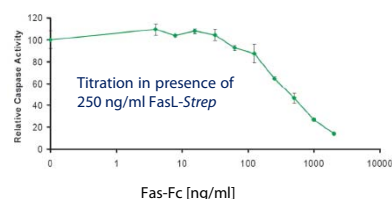
## Homogeneous FasL-*Strep* Apoptosis Assay

The homogeneous FasL-*Strep* Apoptosis Assay can be performed in the presence of the anti-*Strep*-tag® antibody "*Strep*MAB-Immo" which increases 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 *Strep*MAB-Immo.



Apoptotic activity of FasL-*Strep* is enhanced by cross-linking via the monoclonal antibody *Strep*MAB-Immo



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

## Acknowledgement

IBA thanks the whole team of Apogenix GmbH, Heidelberg, for substantial support.

The data of this flyer are from "Kleber et al., 2008, Cancer Cell 13, 235-248".

## Order information

Cat. No.	Description	Amount
2-3911-000	Heterogeneous FasL- <i>Strep</i> Apoptosis Assay, human	96 assays
2-3912-000	Homogeneous FasL- <i>Strep</i> Apoptosis Assay, human	96 assays
2-3961-000	Heterogeneous FasL- <i>Strep</i> Apoptosis Assay, murine	96 assays
2-3962-000	Homogeneous FasL- <i>Strep</i> Apoptosis Assay, murine	96 assays
2-3901-010	Human FasL- <i>Strep</i>	10 µg (4x 2.5 µg)
2-3901-002	Human FasL- <i>Strep</i>	2.5 µg
2-3951-010	Mouse FasL- <i>Strep</i>	10 µg (4x 2.5 µg)
2-3951-002	Mouse FasL- <i>Strep</i>	2.5 µg
2-1517-001	<i>Strep</i> MAB-Immo; purified	100 µg
2-1501-001	<i>Strep</i> -Tactin® coated microplate	1 plate
2-1501-050	<i>Strep</i> -Tactin® coated microplates	5 plates

The Fas ligand:T4-FOLDON fusion technology and/or its use is subject to patent applications owned by Apogenix GmbH, Heidelberg.

*Strep*-tag® and *Strep*-Tactin® are covered by patents and patent applications owned by IBA.

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