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ProductInformation

Fas Ligand Set

Product Code **F4428**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonyms
FasL; Apo-1L; CD95L; CD178; TNFSF 6

Product Description
Human Fas Ligand (amino acid residues 133-281) is expressed in HEK (human embryonic kidney) -293 cells as an N-terminal FLAG tagged protein. It migrates on SDS-PAGE as a triplet of approximately 26-32 kDa due to different degrees of glycosylation. This FLAG tagged FasL requires the presence of FLAG antibody for its aggregation and activation.

Fas ligand, a protein belonging to the tumor necrosis factor (TNF) family of cytokines, induces apoptosis in cells expressing the cell membrane receptor Fas (CD95/Apo-1). FasL is expressed mainly in lymphoid tissue such as spleen and thymus and in a few other non-lympoid tissues including the eye, testis, and thyroid.[1,2]

FasL is involved in immune down regulation and in the process of negative selection of T cells in the peripheral lymphoid organs. It also plays an important role in conferring immuno-privilege to the non-lymphoid tissues, such as eye and testis, in which it is expressed. It is suggested that constitutive FasL expression in a variety of tumors contributes to their immune evasion.[3-4] FasL is considered a candidate for anticancer chemotherapy since many tumor types express its receptor, Fas. This application is limited to local administration due to the fact that many organs, such as liver, lung and heart express Fas.[5]

Recently it was shown that FasL is also involved in mediating inflammatory processes and can have pro-proliferative effects. The dual activity of FasL can be explained by diversion of the FasL signal to apoptosis of proliferation/differentiation, down stream of its receptor.[6-7]

Reagents Provided
- **Fas Ligand**, Product Code F4178 10 µg
  Human, recombinant, N-terminal FLAG-tagged, expressed in HEK-293 cells.
  Solution in PBS, 0.2 µm filtered, endotoxin tested
  Purity: >95%

- **ANTI-FLAG® M2**, Product Code F3165 0.2 mg
  Mouse monoclonal antibody

Reagents and Equipment Required But Not Provided
(Sigma product numbers are given where appropriate)
- Jurkat cells
- Jurkat cell growth medium: RPMI (R8758), 10% heat inactivated FCS (F2442), 1:100 Pen/Strep solution (P0781), 10 mM Glutamine (G7513)
- Dulbecco's Phosphate Buffered Saline (PBS, D8537)
- Caspase-3 assay kit (CASP-3-F)
- 17 megohm water
- 50 ml disposable tube
- Four 15 ml disposable tube
- Two PCR tube strips - 0.2 ml
- 96 well plate, flat bottom
- 96 well plate, V shape
- Multichannel pipettes – 0.5-10 µl, 5-50 µl, and 40-250 µl
- Sterile tips – 10 µl, 200 µl
- 96 well plate for fluorimeter, white or equivalent
- Fluorimeter – plate reader
- Centrifuge

Storage
Store the kit at –20 °C. When stored unopened, the components in this kit are stable for 2 years

Procedure

Assay principle

The assay is based on induction of apoptosis in Jurkat cells by FasL, leading to activation of the caspase cascade. The assay is performed in the presence of ANTI-FLAG M2 antibody that activates the soluble FLAG tagged FasL by inducing its aggregation. The extent of apoptosis induction is determined by measuring the activity of caspase-3 using a CASP-3-F kit.

The test is performed in a high-throughput format in a 96 well plate.

Note: This assay procedure is an example using Jurkat cells. When other cell lines are used, the concentration of Fas Ligand and ANTI-FLAG M2 required may be different and may need calibration.

Assay

1. Collect 2-3 x 10^7 Jurkat cells by centrifugation and resuspend the cells in Jurkat growth medium at approximately 2-3 x 10^6 cell per ml in approximately 10 ml
2. Dispense 2 ml of cell suspension in 4 x 15 ml centrifuge tubes. Keep 1-2 ml of cell suspension for a blank.
3. Dilute the ANTI-FLAG M2 antibody to 0.5 mg/ml with PBS.

Table 1.

Final Ab concentration, µg/ml	**0**	**0.25**	**0.5**	**1**
Jurkat cell suspension, ml	2	2	2	2
ANTI-FLAG M2 0.5 mg/ml, µl	0	1	2	4

4. Add ANTI-FLAG M2 to the cell suspension tubes according to Table 1. Mix gently.
5. Using PCR tube strips, dilute Fas Ligand 100, 500, 1,000 and 2,000-fold in Jurkat growth medium.
6. Dispense 100 µl of cell suspension into the appropriate wells of a 96 well plate (see Table 2). Dispense 100 µl of cells without antibody into the blank (Blk) row.
7. Add 1 µl of Fas Ligand from each dilution into the appropriate wells using a 0.5-10 µl multichannel pipette. Mix by pipeting and change tips after each sample addition.

Table 2.
Reaction scheme

	No Ab				0.25 µg/ml Ab				
	1	2	3	4	5	6	7	8	9
FasL dilution	100	500	1000	2000	100	500	1000	2000	
A	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk
B	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk
C	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk
	0.5 µg/ml Ab				1 µg/ml Ab				
FasL dilution	100	500	1000	2000	100	500	1000	2000	
D	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk
E	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk
H	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	Blk

8. Incubate 96 well plates in the CO_2 incubator for 3-4 hours.
9. Suspend cells by pipeting and transfer the cells to a 96 well V-shaped plate.
10. Collect cells by centrifugation for 5 minutes at 600 x *g*.
11. Remove the supernatant carefully using a multichannel pipette.
12. Place the 96 well plates with cells on ice.

Caspse-3 activity determination

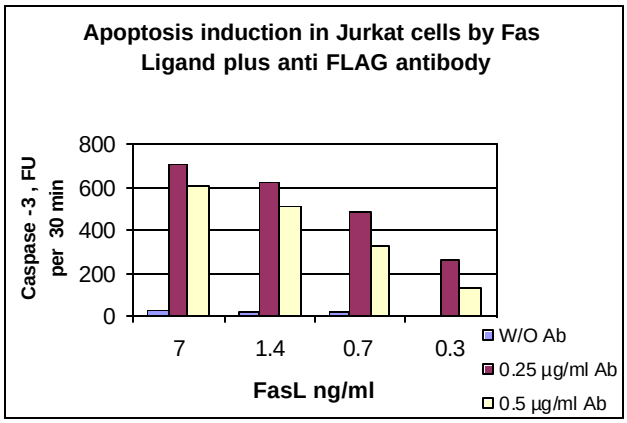
1. Dilute 5X Lysis buffer, 5-fold using 17 megohm water.
2. Add 25 µl of lysis buffer to each well. Use a multichannel pipette.
3. Pipet up and down to suspend cells.
4. Leave on ice for 10-20 minutes.
5. In the meantime turn on the fluorimeter.
   - Set parameters: Excitation = 360 nm, Emission = 440 nm, Slit width = 5 nm
   - Program: well plate reader, kinetics, 10 cycles, every 300 sec (5 min), 1 sec per well.

   Note: cells other than Jurkat may need longer time periods for caspase-3 activity detection
6. Calculate the volume of 1X assay buffer needed: 200 µl per well + 2-3 ml extra, (for 60 wells prepare 15 ml 1X assay buffer).
7. Dilute 10X Assay buffer, 10-fold with 17 megohm water
8. Add 5 µl of substrate per 3 ml of 1X assay buffer (25 µl per substrate per 15 ml 1X assay buffer).
9. Add 200 µl of 1X Assay buffer containing substrate to each well using multichannel pipette.
10. Pipet to mix and transfer 200 µl of each sample to a 96 well fluorimeter plate.
11. Read the fluorescence.
12. Calculate the difference of the fluorescence intensity per time period.

Results

Activity: 0.5-5 ng/ml FasL induces apoptosis in Jurkat cells in the presence of 0.25-1 µg/ml ANTI-FLAG M2 Monoclonal antibody as measured by caspase-3 activity.

Product Profile



References

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2. Guller, S., Ann. NY Acad. Sci., **828**, 268-272 (1997).
3. Sakata, K., et al., Clin. Immunol. Immunopath., **87**, 1-7 (1998).
4. O'Connel, J., et al., Immunology Today, **20**, 47-52 (1999).
5. Poulaki, V., et al., Drug Resist. Updat., **4**, 233-242 (2001).
6. Budd, R.C., J. Clin. Invest., **109**, 437-442 (2002).
7. Hohlbaum, A.M., Clin. Immunol., **103**, 1-6 (2002).

Related Products

Monoclonal Anti-Fas Ligand, Clone Alf2.1	F 2051
Monoclonal Anti-Fas Ligand, Clone Alf1.2	F 1926
Fas Ligand PCR Primer Set	F 8300
Monoclonal Anti-Fas, Clone DX2	F 4424
Monoclonal Anti-Fas, Clone DX2-FITC	F 4229

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