

These caspases are responsible for the cleavage of the key cellular proteins that leads to the typical morphological changes observed in cells undergoing apoptosis. TRAIL R3 also called DcR1 (Decoy Receptor 1) and TRAIL R4 called TRUNDD (TRAIL Receptor with a truncated death domain) or DcR2 (Decoy Receptor 2) are unable to initiate apoptosis because of the lack of a functional death domain.

### Granzyme /Perforin pathway

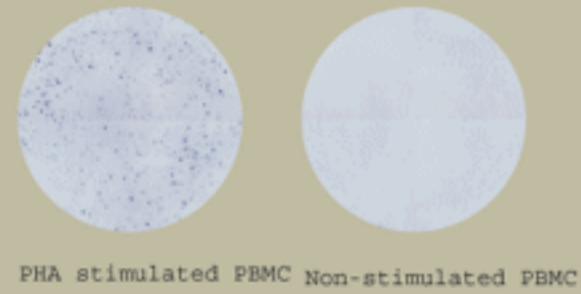
One mechanism used by cytotoxic T cells to kill tumor cells and virus-infected cells is the release of granzyme and perforin (a membrane pore forming protein) proteins.

Following adhesion of the cytotoxic cell to the target, granzyme B is directly exocytosed and enters the target cell, assisted by perforin. Perforin polymerises into transmembrane pores facilitating the intracellular delivery of the granzymes. Granzyme B induces cleavage of procaspase-8 which activates a caspase cascade and cleavage of factors like ICAD that results in the apoptotic death of the cell, and interaction of granzyme B with other targets can invoke mitochondrially mediated death pathways.

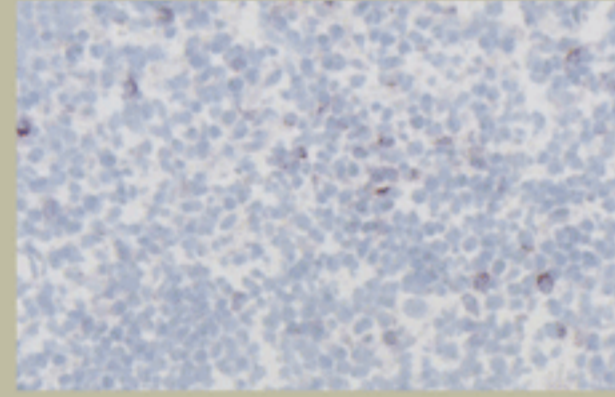
Diaclone also offers Granzyme B Elisa and Elispot.

ELISA Kits to human Granzyme B				
Size (tests)	Cat n°	Range	Sensitivity	
1x96	850 790 096	31.25 pg/ml - 1 000 pg/ml	20 pg/ml	
2x96	850 790 192			

ELISPOT Kits to human Granzyme B				
Size (tests)	Without plates	With PVDF plates		
		Non-sterile	Sterile	
	/	856 131 001	856 131 111	856 131 111
5x96	856 131 005	856 131 005 P	856 131 005 S	856 131 005 S
10x96	856 131 010	856 131 010 P	856 131 010 S	856 131 010 S
15x96	856 131 015	856 131 015 P	856 131 015 S	856 131 015 S
20x96	856 131 020	856 131 020 P	856 131 020 S	856 131 020 S



PHA stimulated PBMC Non-stimulated PBMC



Immunohistochemical staining of perforin in human tonsil paracortex with anti perforin, clone B-D48 (Cat N°854 950 000).

Courtesy of Dr. J.A. Kummer, University Medical Center, Utrecht

Monoclonal Antibodies to human perforin					
Clone	Isotype	Format	Application	Cat n°	Size
B-D48	Mouse IgG1	Purified	FCM	854 953 020	200 tests / 2ml
		Azide free	IHC (F),WB	854 950 000	200 µg / 200µl
		PE	FCM	854 952 010	100 tests / ml

FCM = Flow Cytometry ; IHC (F) = Immunohistochemistry (frozen) ; WB = Western Blot

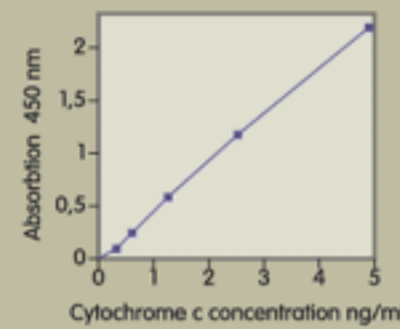
Flow cytometric analysis of perforin in human peripheral blood lymphocytes (A) and YT-1 cell line (B). Cells were stained with anti perforin-PE monoclonal antibody, clone B-D48 (Cat N°854 952 010) and an anti IgG1 PE isotype control, clone B-Z1 (Cat N° 857 072 010)

Because of the importance and specificity of Granzyme B and perforin for cytotoxic cells, Diaclone is proud to introduce its new monoclonal antibody to human perforin.

### Role of Mitochondria in apoptotic signalling

Mitochondria participate in apoptotic signalling pathways through the release of mitochondrial proteins into the cytoplasm. Cytochrom c, a key protein in electron transport is released from mitochondria in response to apoptotic signals and together with Apaf-1 and ATP forms a complex with procaspase-9 required for formation of the Apoptosome leading to activation of caspase 9 and the caspase cascade. Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-X<sub>L</sub> prevent pore formation and act as a guardian of mitochondrial integrity controlling the release of mitochondrial proteins into the cytoplasm.

AIF (Apoptosis inducing factor) is another mitochondrial factor that is released into the cytoplasm to induce apoptosis in a caspase independent way.



ELISA Kits to human Cytochrom c				
Size (tests)	Cat n°	Range	Sensitivity	
1x96	650 070 096	0.08 ng/ml - 5 ng/ml	0.08 ng/ml	

### The p53 signalling pathway

P53 is a tumor suppressor protein which is activated as a transcription factor in response to DNA damage, hypoxia or oncogene activation resulting in growth arrest and / or apoptosis. Cell cycle arrest allows DNA repair to proceed before mitosis if the damage can not be repaired; p53 activation results in apoptosis. The critical role of p53 is evidenced by the fact that it is mutated in more than 50% of all human cancers.

Monoclonal Antibodies to human P53						
Description	Clone	Isotype	Species	Format	Application	Size
P53	B-P3	Mouse IgG2a	Human	Azide free	FCM, IP, WB, IHC (F+P)	200 tests / 2 ml

FCM = Flow Cytometry; IHC (F) = Immunohistochemistry (frozen); IHC (P) = Immunohistochemistry (paraffin); IP = Immunoprecipitation; WB = Western Blot

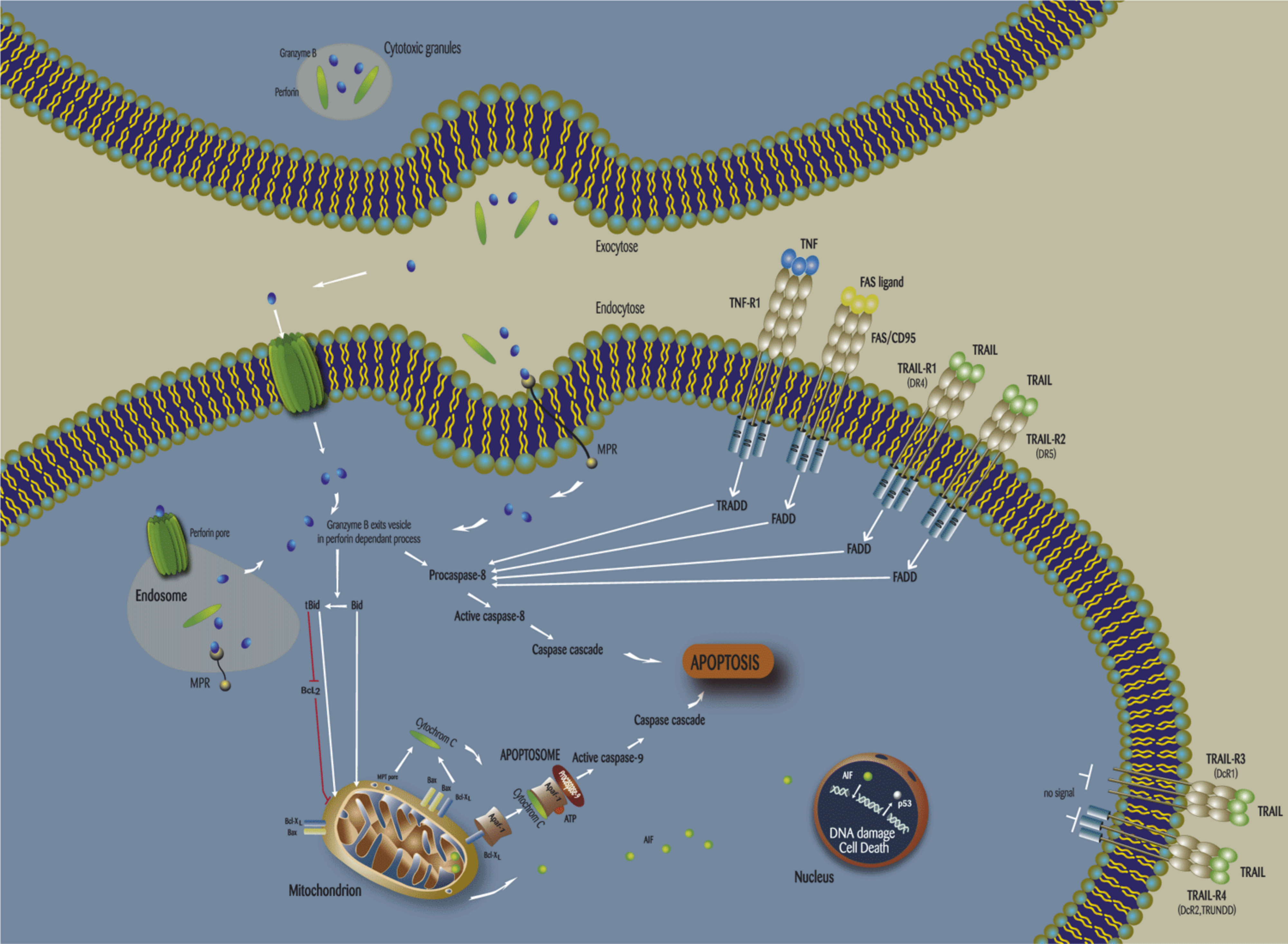
ELISA Kits to human P53				
Size (tests)	Cat n°	Range	Sensitivity	
1x96	850 630 096	3.1 U/ml - 100 U/ml	1.5 U/ml	
2x96	850 630 192			



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# Apoptosis



## APOPTOSIS TOOLS

Apoptosis or programmed cell death, is a normal component of the development and health of multicellular organisms. The term apoptosis had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr, Wyllie and Curie (Brit. J. Cancer 26: 239) in 1972. Apoptosis is of a Greek origin, having the meaning "falling off or dropping off" in analogy to leaves falling off trees or petals dropping off flowers.

Apoptosis is distinct from another form of cell death called necrosis which is associated with acute injury to cells, resulting in a loss of membrane integrity, swelling and disruption of the cells. During necrosis, the cellular contents are released uncontrolled into the cell's environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue.

Apoptosis, by contrast is a process in which cells play an active role in their own death. Apoptotic cells can be recognized by several morphological changes: staging membrane budding, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation followed by rapid engulfment of corpses by neighbouring cells. Apoptosis can be triggered by extrinsic and intrinsic signals. These various stimuli include UV or  $\gamma$ -irradiation, treatment with cytotoxic drugs, over-expression of tumor-suppressor genes, growth factor withdrawal or activation of death receptors.

## Death Receptors and Decoy Receptors

DIACLONE Research has developed monoclonal antibodies, ELISA and Elispot immunoassays to better understand apoptosis and related diseases.

### ELISA Kits to TNF ligands and receptors

Specificity	Size (tests)	Cat N°	Species	Range	Sensitivity
Fas / CD95	1x96	850 740 096	Human	93.75 pg/ml - 3 000 pg/ml	47 pg/ml
	2x96	850 740 192			
Fas Ligand / CD178	1x96	850 750 096	Human	62.5 pg/ml - 2 000 pg/ml	44 pg/ml
	2x96	850 750 192			
TNF- $\alpha$	1x96	850 090 096	Human	25 pg/ml - 800 pg/ml	8 pg/ml
	2x96	850 090 192			
TNF- $\alpha$ CE	1x96	950 090 096	Human	25 pg/ml - 800 pg/ml	8 pg/ml
	2x96	950 090 192			
TNF- $\alpha$	1x96	860 040 096	Mouse	31.25 pg/ml - 1 000 pg/ml	25 pg/ml
	2x96	860 040 192			
TNF- $\alpha$	1x96	865 000 096	Rat	31.25 pg/ml - 1 000 pg/ml	10 pg/ml
	2x96	865 000 192			
TRAIL / APO2L	1x96	850 770 096	Human	93.75 pg/ml - 3 000 pg/ml	64 pg/ml
	2x96	850 770 192			
TRAIL R1 / DR4	1x96	850 820 096	Human	31.25 pg/ml - 1 000 pg/ml	8 pg/ml
	2x96	850 820 192			
TRAIL R2 / DR5	1x96	850 830 096	Human	31.25 pg/ml - 1 000 pg/ml	6 pg/ml
	2x96	850 830 192			
TRAIL R3 / DcR1	1x96	850 840 096	Human	312.5 pg/ml - 1 0000 pg/ml	147 pg/ml
	2x96	850 840 192			
TRAIL R4 / DcR2 / TRUNDD	1x96	850 850 096	Human	312.5 pg/ml - 1 0000 pg/ml	64 pg/ml
	2x96	850 850 192			

### ELISPOT Kits to TNF $\alpha$

Species	Size (tests)	Without plates		
		Non-sterile	Sterile	
Human	1x96	/	856 041 001	856 041 111
	5x96	856 041 005	856 041 005 P	856 041 005 S
	10x96	856 041 010	856 041 010 P	856 041 010 S
	15x96	856 041 015	856 041 015 P	856 041 015 S
	20x96	856 041 020	856 041 020 P	856 041 020 S
	1x96	/	867 021 001	867 021 111
Rat	5x96	867 021 005	867 021 005 P	867 021 005 S
	10x96	867 021 010	867 021 010 P	867 021 010 S
	15x96	867 021 015	867 021 015 P	867 021 015 S
	20x96	867 021 020	867 021 020 P	867 021 020 S

### Monoclonal Antibodies to TNF ligands and receptors

Description	Clone	Isotype	Species	Format	Application	Size	Cat N°
CD40	B-820	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	854 283 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 280 000
				FITC	FCM	100 tests / ml	854 281 010
				PE	FCM	100 tests / ml	854 282 010
CD40 Ligand / CD154	B-829	Mouse IgG2a	Human	Purified	FCM, IHC, (F)	200 tests / 2 ml	854 293 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 290 000
				FITC	FCM	100 tests / ml	854 291 010
				PE	FCM	100 tests / ml	854 292 010
Fas / CD95	B-D29	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	853 033 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	853 030 000
	B-G27	Mouse IgG2a	Human	Purified	FCM, IHC, (F)	200 tests / 2 ml	853 003 020
				Azide free	BA, IHC, (F)	200 $\mu$ g / 200 $\mu$ l	853 000 000
Fas Ligand / CD178	B-R17	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	853 073 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	853 070 000
				Biotin	FCM	100 tests / ml	853 074 010
				PE	FCM	100 tests / ml	853 072 020
Anti TNF- $\alpha$	B-C7	Mouse IgG1	Human	Azide free	BA	0,5 mg	855 150 005
				FITC	FCM	100 tests / ml	855 161 019
	B-D9	Mouse IgG1	Human	PE	FCM	100 tests / ml	855 162 019
				Purified	FCM	200 tests / 2 ml	853 083 020
Anti TRAIL / APO2L	B-S23	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	853 083 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	853 080 000
				PE	FCM	100 tests / ml	853 082 010
				Biotin	FCM	100 tests / ml	853 084 010
Anti TRAIL R1 / DR4	B-N36	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	854 853 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 850 000
				PE	FCM	100 tests / ml	854 852 010
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	853 093 020
Anti TRAIL R2 / DR5	B-K29	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	854 863 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 860 000
				PE	FCM	100 tests / ml	854 862 010
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 870 000
Anti TRAIL R3 / DcR1	B-D44	Mouse IgG1	Human	Purified	FCM	200 tests / 2 ml	854 893 020
				Azide free	BA	200 $\mu$ g / 200 $\mu$ l	854 890 000
				PE	FCM	100 tests / ml	854 892 010

BA = Biological Assay; FCM = Flow Cytometry; IHC (F) = Immunohistochemistry (frozen)

Extrinsic apoptosis signalling is mediated by the activation of so-called "death receptors" which are cell surface receptors that transmit apoptotic signals after ligation with specific ligands. Death receptors belong to the tumor necrosis factor (TNF) gene superfamily, including CD95 (or Fas), TNFR1 and the TRAIL (TNF-related apoptosis inducing ligand) receptors DR4 (TRAIL R1) and DR5 (TRAIL R2). All members of the TNFR family possess a cysteine-rich extracellular domain and an intracellular conserved sequence called death domain (DD). Binding of their ligands results in the trimerization and activation of the respective receptors. Adapter molecules like FADD or TRADD possess their own DDs by which they are recruited to the DDs of the activated death receptor to form the death inducing signalling complex: DISC. This results in the activation of an initiator caspase such as caspase-8 or caspase-10. These caspases can then activate other caspases in a cascade.