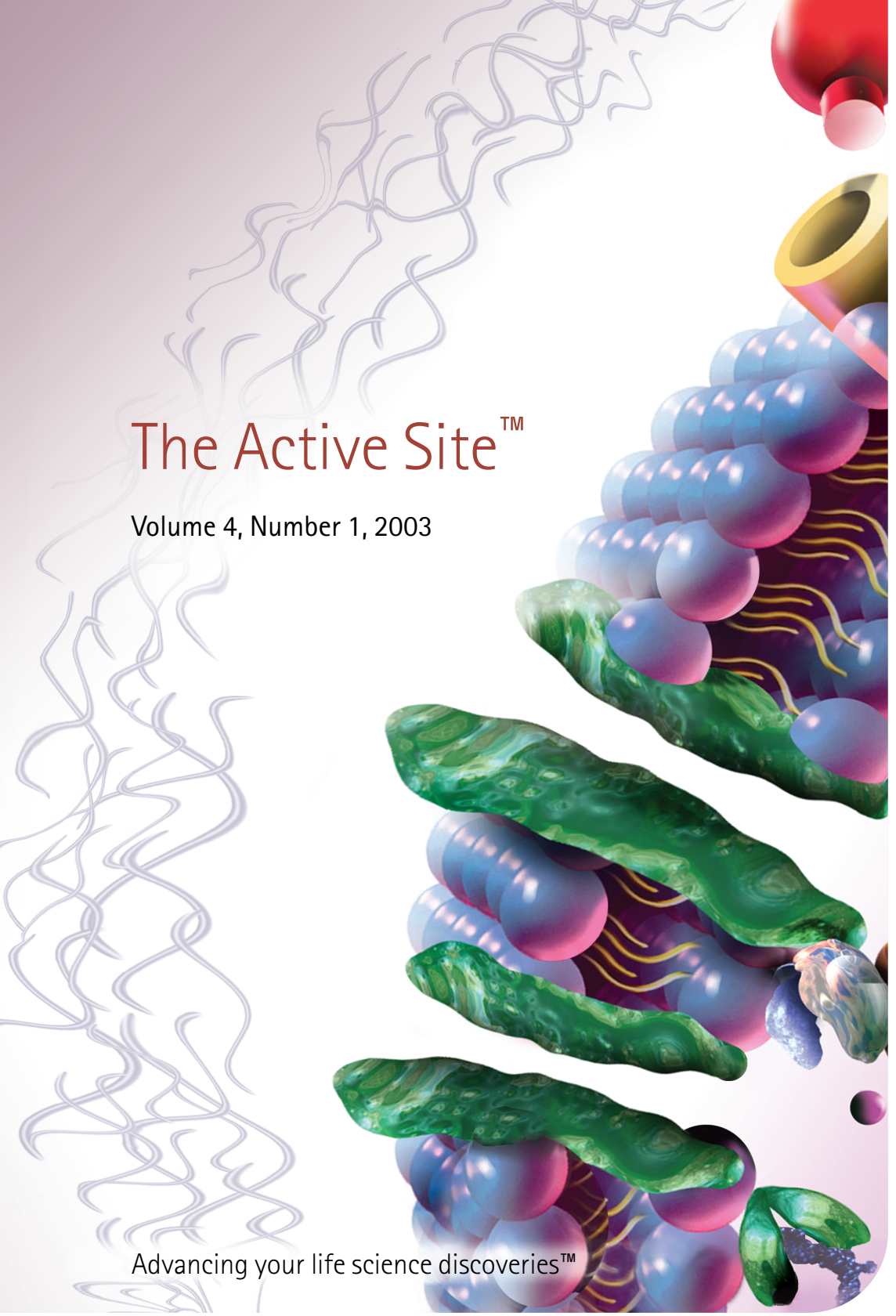

The Active Site™

Volume 4, Number 1, 2003

Advancing your life science discoveries™

oncogene™ RESEARCH PRODUCTS

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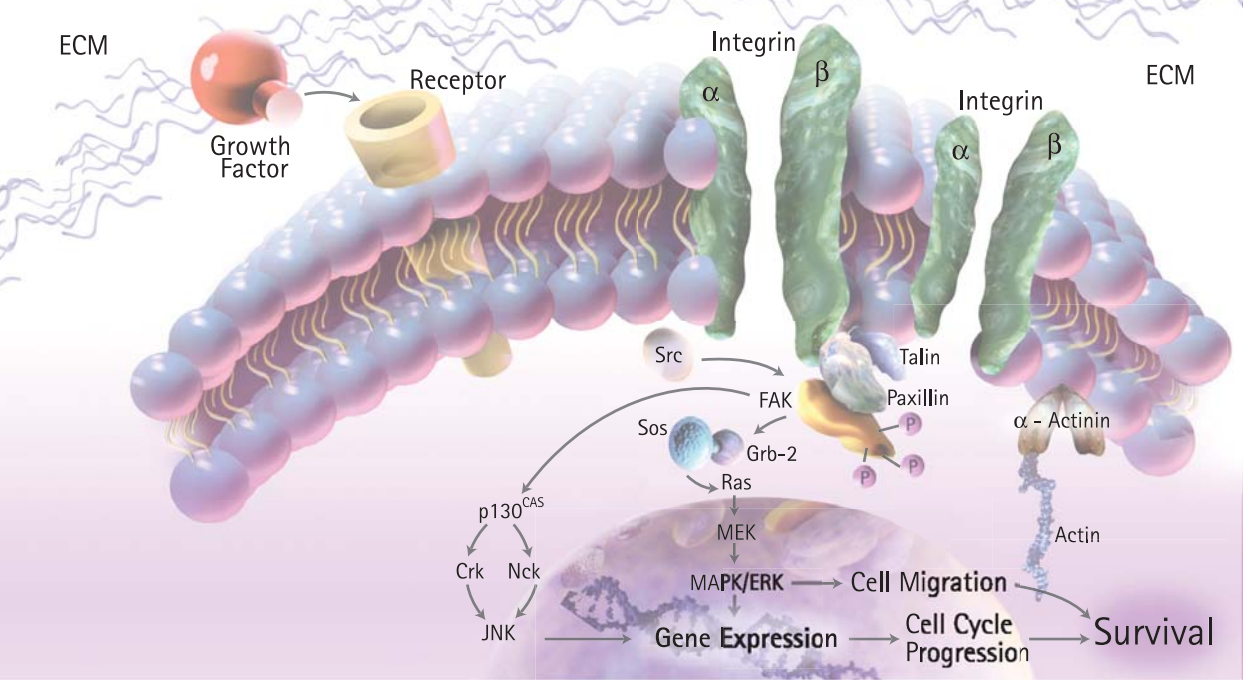
EMD

Integrins:
Molecular Adhesives in Cell Survival & Cell Death

The ability of cells to adhere to other cells and to the extracellular matrix (ECM) is important for growth, development, apoptosis, inflammation, migration, tumorigenesis, and immune responses. Amongst all the various molecules involved in the adhesion process, integrins are considered as the most important group. While generally classified as adhesion molecules, integrins also play an important role in signal transduction. Signal transduction through integrins occurs in two directions - moving from the extracellular microenvironment into the cell (outside-in signaling) and from the cytoplasm to the extracellular domain of the receptor (inside-out signaling).

Integrins are heterodimers composed of a variable α-subunit of 150-170 kDa and a conserved 95 kDa β-subunit. They contain a large extracellular domain responsible for ligand binding, a single transmembrane domain, and a cytoplasmic domain. The exact combination of various α- and β-subunits dictates the binding specificity of integrins to different ECM components. Although both subunits are required for adhesion, the binding specificity primarily depends on the extracellular region of the α-subunit. The structural similarities between α-subunits of various integrins are remarkable. Their extracellular domains contain seven homologous repeats of 30 to 40 amino acids spaced by stretches of 20-30 amino acids. The three or four repeats, which are in the extreme extracellular region, are involved in ligand binding. The α-subunits of all integrins share the five amino acid motif, GFFKR, located directly under the transmembrane region. The exact function of this motif has not been elucidated. The β-subunits contain tandem repeats of four cysteine-rich regions that are essential for maintaining the tertiary structure of integrins.

The recognition site for most integrins that bind the ECM consists of an RGD (arginine-glycine-aspartic acid) sequence. Integrins bind to their ligands with low affinity and this binding occurs only when a certain minimum number of integrins are present at specific points known as focal contacts. In resting cells integrins are diffused over the cell surface and lack sufficient adhesive force. In response to specific stimuli they cluster in focal contacts and their combined affinities create a region on the cell surface, which presents sufficient adhesive capacity to adhere to the ECM. This allows cells to bind to a large number of matrix molecules simultaneously while maintaining their ability to explore their environment without losing all attachments. Any stronger binding to their ligands will cause an irreversible binding to the matrix, depriving them of their motility. Upon binding to ECM molecules, integrins undergo a conformational change that allows the intracellular domain of their β-subunit to interact with focal-adhesion proteins such as talin and α-actinin.



Integrin-mediated cell attachment is essential for survival signaling in many types of normal cells and offers protection against a variety of apoptotic stimuli. Epithelial and endothelial cells that are largely dependent on integrin-mediated cell attachment for their survival undergo apoptosis upon loss of integrin-mediated cell attachment. Although integrins do not possess any enzymatic activity or a kinase domain, they initiate signaling through association with non-receptor kinases, such as focal adhesion kinase (FAK) and the Src family of kinases. These kinases in turn activate several downstream survival pathways, such as PI 3-kinase, Akt, and MAPK/ERK. Integrins interact with a number of regulatory adaptor molecules, such as paxillin, and with the structural proteins, vinculin and talin, that couple integrin to the actin cytoskeleton. Upon integrin ligation to the ECM, paxillin associates with FAK and induces a conformational change that facilitates FAK tyrosine phosphorylation and its activation. Upon activation, FAK combines with Src, which phosphorylates paxillin and $p130^{CAS}$. Both paxillin and $p130^{CAS}$ serve as scaffolds for the recruitment of various adaptors and signaling intermediates.

FAK does not contain an SH2 or SH3 domain; hence it localizes at focal adhesions by binding to talin and paxillin via its C-terminal region, known as the Focal Adhesion Targeting (FAT) sequence. FAK contains several tyrosine residues that are phosphorylated in response to growth factors and cell spreading. FAK autophosphorylates on Tyr^{39}, which allows it to bind to the SH2 domain of Src. Src phosphorylates a number of tyrosine residues on FAK, particularly $Tyr^{567, 576}$ that are important for regulation of its kinase activity. Src also phosphorylates Tyr^{925}, which serves as a docking site for the SH2 domain of Grb2 and links integrin signaling to ERK activation. FAK also contains two proline-rich regions that bind to the SH3 domain of $p130^{CAS}$, a large adaptor protein that can bind to other adaptor proteins such as Crk and Nck. Phosphorylation of tyrosine residues on $p130^{CAS}$ by the FAK-Src complex allows it to serve as a docking site for the SH2 domains of Crk and Nck. In addition to phosphorylating FAK and $p130^{CAS}$, Src also phosphorylates paxillin and tensin, which are involved in regulating the focal adhesion.

It is well known that loss of adhesion from the ECM can trigger apoptosis in normal cells and that cell detachment is an essential step in tumor metastasis and malignancy. Also, integrin-mediated cell attachment has been shown to modulate cancer cell responses to chemotherapeutic agents. Hence, elucidation of the molecular mechanisms contributing to defective adhesion is important in developing new drugs for treatment of the resulting diseases.

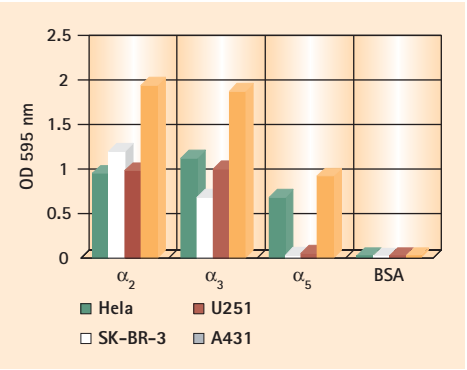
References:

Hsia, D.A., et al. 2003. *J. Cell Biol.* **160,** 753.
Lewis, J. M. et al. 2002. *Proc. Natl. Acad. Sci. USA* **99,** 3627.
Wade, R., et al. 2002. *Oncogene* **21,** 96.
Aoudjit, F., and Vuori, K. 2001. *Oncogene* **20,** 4995.
Zamir, E., and Geiger, B. 2001. *J. Cell Sci.* **114,** 3583.
Giancotti, F.G. 2000. *Nat. Cell Biol.* **2,** E13.
Gilmore A.P., et al. 2000. *J. Cell Biol.* **149,** 431.
Damiano, J.S., et al. 1999. *Blood* **93,** 1658.
Etzioni, A. 1999. *Lancet* **353,** 341.
Liu, S., et al. 1999. *Nature* **402,** 676.
Hemler M.E. 1998. *Curr. Opin. Cell Biol.* **10,** 578.
Schlaepfer, D.D., et al. 1998. *Mol. Cell. Biol.* **18,** 2571.
Vuori, K. 1998. *J. Membr. Biol.* **165,** 191.
Frisch, S.M., and Ruoslahti, E. 1997. *Curr. Opin. Cell Biol.* **9,** 701.
Hungerford, J.E., et al. 1996. *J. Cell Biol.* **135,** 1383.
Wary, K.K., et al. 1996. *Cell* **87,** 733.
Hynes, R.O. 1992. *Cell* **69,** 11.

Integrin Detection Kits

The Integrin Detection Kits utilize murine monoclonal antibodies to integrin subunits α_2, α_3, and α_5 to bind to the integrins on the cell surface. The antibodies are immobilized on a plastic surface. The assay is specific and sensitive; only cells with the relevant integrins attach to the antibody-coated wells, which can be quantitated by incubating with a cell staining solution.

α_2, α_3, α_5 Integrin Detection Kit



Format: 192 Tests Assay Time: 3.5 Hours

Sample Type: Cultured cells

Comments: Suitable for the determination of the relative concentrations of integrin α_2, α_3, and α_5.

Kit Contains: Antibody-coated microplate strips, anti-α_2 integrin IgG, anti-α_3 integrin IgG, anti-α_5 integrin IgG, antibody diluent, cell staining solution, extraction buffer, and a directional insert.

Cat. No. QIA121 1 Kit

α_2 Integrin Detection Kit

Format: 96 Tests Assay Time: 2.5 Hours Sample Type: Cultured cells

Comments: Suitable for the determination of relative concentrations of α_2 integrins.

Kit Contains: Antibody-coated microplate strips, control microplate strips, anti-α_2 integrin IgG, antibody diluent, cell staining solution, extraction buffer, and a directional insert.

Cat. No. QIA122 1 Kit

α_3 Integrin Detection Kit

Format: 96 Tests Assay Time: 2.5 Hours Sample Type: Cultured cells

Comments: A convenient kit for the determination of the relative concentrations of α_3 integrins.

Kit Contains: Antibody-coated microplate strips, control microplate strips, anti-α_3 integrin IgG, antibody diluent, cell staining solution, extraction buffer, and a directional insert.

Cat. No. QIA123 1 Kit

α_5 Integrin Detection Kit

Format: 96 Tests Assay Time: 2.5 Hours Sample Type: Cultured cells

Comments: A convenient kit for the determination of the relative concentrations of α_5 integrin.

Kit Contains: Antibody-coated microplate strips, control microplate strips, anti-α_5 integrin IgG, antibody diluent, cell staining solution, extraction buffer, and a directional insert.

Cat. No. QIA124 1 Kit

Antibodies to Integrins

Product	Cat. No.	Comments	Size
Anti-Integrin α_{IIb}, Human (Mouse)	407313	Immunogen used was human M21-L-IIb melanoma cells. Recognizes the human α_{IIb} integrin chain. **ELISA, IH**	100 µl
Anti-Integrin α_v (Ab-1), Human (Mouse)	407286	Immunogen used was human placental $\alpha_v\beta_3$. Recognizes all α_v integrins. Blocks cell adhesion mediated by all α_v integrins and *in vivo* melanoma growth and angiogenesis. **ELISA, FC, IP, PS**	100 µg
Anti-Integrin α_v (Ab-2), Human (Mouse)	407287	Immunogen used was human M21 melanoma cells. Recognizes all α_v integrins. Blocks $\alpha_v\beta_6$ and $\alpha_v\beta_1$ function. **ELISA, IP, PS**	100 µl
Anti-Integrin α_v (Ab-3), Human (Mouse)	407288	Immunogen used was human M21 melanoma cells. Cross-reacts with all α_v integrins. Blocks $\alpha_v\beta_6$ and $\alpha_v\beta_1$ function. **ELISA, FC, IP, PS**	100 µl
Anti-Integrin α_v (Ab-4), Human (Mouse)	407289	Immunogen used was human M21 melanoma cells. Cross-reacts with all α_v integrins. Blocks $\alpha_v\beta_6$ and $\alpha_v\beta_1$ function. **ELISA, FC, IP, PS**	100 µl
Anti-Integrin α_v (Ab-5), Human (Mouse)	407311	Immunogen used was α_v integrin from human M21 melanoma cells. Cross-reacts with all α_v integrins. Blocks $\alpha_v\beta_6$, $\alpha_v\beta_5$, and $\alpha_v\beta_1$ function. **ELISA, FC, IB, PS**	100 µl
Anti-Integrin α_v (Ab-6), Human (Mouse)	407312	Immunogen used was human M21 melanoma cells. Reacts with all α_v integrins. Blocks $\alpha_v\beta_6$, $\alpha_v\beta_5$, and $\alpha_v\beta_1$ function. **ELISA, FC, PS**	100 µl
Anti-Integrin β_3 (Ab-1), Human (Mouse)	407314	Immunogen used was human M21 melanoma cells. Cross-reacts with $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin chains. **ELISA, FC, IB, IP, PS**	100 µl
Anti-Integrin β_3 (Ab-2), Human (Mouse)	407315	Immunogen used was human M21-L-IIb melanoma cells. Cross-reacts with $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin chains. **ELISA, FC, IB, IP, PS**	100 µl
Anti-Integrin β_5, Human (Mouse)	407316	Immunogen used was human UCLA-P3 lung carcinoma cells. Reacts with human β_5 integrin chain. **ELISA, FC, IB, IP, PS**	100 µl
Anti-Integrin β_6, Human (Mouse)	407317	Immunogen used was $\alpha_v\beta_6$ human recombinant protein. Reacts with human β_6 integrin chain. **ELISA, FC, IB, IP, PS**	100 µl

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **IB:** Immunoblotting; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

Anti-Pleckstrin, C-Terminal, Human (Rabbit)

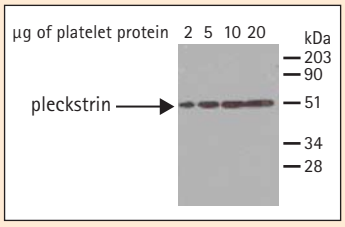
A synthetic peptide corresponding to amino acids 337-350 of pleckstrin was used as an immunogen.

Detects the ~47 kDa pleckstrin, the major substrate of protein kinase C in platelets.

Application: IB

Cat. No. ST1003 50 µl

Ref.: Sloan, D.C., and Haslam, R.J. 1997. *Biochem. J.* **328,** 13.



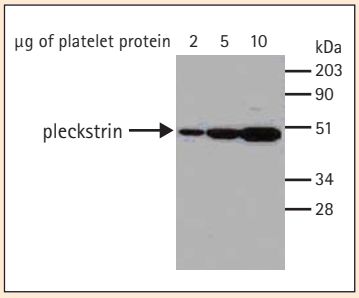
Anti-Pleckstrin, Human (Rabbit)

Purified human pleckstrin was used as an immunogen. Detects only pleckstrin and pleckstrin fragments.

Applications: IB, IP

Cat. No. ST1004 50 µl

Ref.: Sloan, D.C., et al. 2003. *Biochem Biophys. Res. Commun.* **293,** 640.



Paxillin in Focal Adhesion Signaling

Synchronization of adhesion and growth factor signals is made possible by the close proximity of several key molecules in the signaling cascade. Paxillin, a highly conserved 68 kDa multi-domain adaptor molecule, is located at the interface of the plasma membrane and the actin cytoskeleton. In spite of being a relatively small molecule (559 amino acids), paxillin does contain several protein-binding modules which allow it to bind a variety of structural and signaling molecules. Paxillin contains an amino terminus and a carboxyl terminus, which consists of four LIM domains (LIM1-4) arranged in tandem. LIM domains are double zinc-finger motifs of about 50 amino acids, which anchor the protein at the plasma membrane. The exact mechanism of paxillin recruitment to focal adhesions is not understood, however, several paxillin LIM-domain binding partners have been identified and include several kinases and phosphatases.

Paxillin is an important target of tyrosine kinases that are activated as a result of integrin signaling or by growth factor stimulation of cells. FAK in association with Src phosphorylates paxillin at Tyr^{31} and Tyr^{118}. Although tyrosine phosphorylation of paxillin is not absolutely essential for localization to focal adhesions, it does generate functional SH2-binding sites for the Crk family of SH2-SH3 adaptor proteins. These adaptor proteins bind to $p130^{CAS}$. In addition to tyrosine phosphorylation, paxillin is also phosphorylated on $Ser^{188/190}$ at the amino terminal region and at several serines in the LIM domains. This phosphorylation is brought about by PKC as well as by p21-activated kinase (PAK).

Product	Cat. No.	Comments	Size
Anti-Paxillin, Human (Mouse)	CP58	Recombinant full length human paxillin-GST-fusion protein was used as immunogen. Recognizes a 68 kDa protein identified as paxillin in human and rat. Does not cross-react with other cytoskeletal proteins. **IB, IF, IP, PS**	100 µg
Anti-Paxillin, Phospho-Specific (Tyr^{118}), Human (Rabbit)	512880	Recognizes the ~68 kDa paxillin in human and chicken phosphorylated at Tyr^{118} by FAK. **IB, IH**	10 T
Anti-Paxillin, Phospho-Specific (Tyr^{31}), Human (Rabbit)	512882	Recognizes the ~68 kDa paxillin phosphorylated at Tyr^{31} in human and chicken. **IB, IH**	10 T
Anti-Paxillin, Phospho-Specific (Tyr^{181}), Human (Rabbit)	512884	Recognizes the ~68 kDa paxillin in human and chicken phosphorylated at Tyr^{181}. **IB, IH**	10 T

Key: IB: Immunoblotting; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

Note: 1 T = 1 test

Also Available...

Anti-Talin, Human and Rabbit (Mouse)

Cat. No. CP73 100 µg

NEW! Antibodies Against Transcription Factors and Related Proteins

Product	Cat. No.	Comments	Size
Anti-AP-2, Human (Rabbit)	PC692	A synthetic peptide corresponding to amino acid residues 120 - 134 of AP-2 was used as an immunogen. Detects the ~50 kDa transcription factor AP-2 in human. **ELISA, IB**	100 µl
Anti-E2F4 (Ab-2), Human (Mouse)	NA78	E2F4 containing a His•Tag® sequence was used as an immunogen. Detects the ~60 kDa E2F-4 protein in human. **IB**	100 µl
Anti-E2F6, Human (Mouse)	NA79	Full length E2F-6 protein was used as the immunogen. Detects the ~38 kDa E2F-6 protein in human. **IB**	100 µl
Anti-E47, Human (Rabbit)	PC695	A synthetic peptide corresponding to amino acid residues 517 - 531 of E47 was used as an immunogen. Detects the ~75 kDa E47 protein in human. **ELISA, IB**	100 µl
Anti-ESX, Human (Rabbit)	PC624	Full-length human GST-ESX fusion protein overexpressed in *E. coli* was used as an immunogen. Detects a ~42 kDa band in epithelial cell extracts in human. **IB**	100 µg
Anti-c-Ets-1, Human (Rabbit)	PC694	A synthetic peptide corresponding to amino acid residues 201 - 214 of human c-Ets-1was used as an immunogen. Detects the ~54 kDa c-Ets-1 protein in human. **ELISA, IB**	100 µl
Anti-Fra2 (Ab-1), Human (Rabbit)	PC696	A synthetic peptide corresponding to amino acid residues 289 - 305 of Fra2 was used as an immunogen. Detects the ~45 kDa Fra2 protein in human and mouse. **ELISA, IB**	100 µl
Anti-c-Myb, Human (Rabbit)	PC697	A synthetic peptide corresponding to amino acid residues 2 - 16 of c-Myb was used as an immunogen. Detects the 90 kDa c-Myb protein in human. **IB**	100 µl
Anti-NFAT3 (887-902), Human (Rabbit)	PC625	A synthetic peptide corresponding to amino acid residues 887 - 902 of human NFAT3 was used as an immunogen. Detects an ~150 kDa protein representing NFAT3 in HEK293 cells transfected with the human NFAT3 gene. **IB, IC**	100 µg
Anti-NFAT5 (1439-1455), Human (Rabbit)	PC626	A synthetic peptide corresponding to amino acid residues 1439 - 1455 of human NFAT5 was used as an immunogen. Detects an ~170 kDa protein representing NFAT5 in HEK293 cells transfected with the human NFAT5 gene. **IB, IC**	50 µg
Anti-Oct-4, Human (Rabbit)	PC699	Full-length Oct-4 protein was used as an immunogen. Detects the 42 - 45 kDa Oct-4 protein in human. **IB**	100 µl
Anti-Pax3, Human (Rabbit)	CA1010	A GST-Fusion protein containing amino acid residues 1 - 347 of Pax3 was used as the immunogen. Detects Pax3 and Pax3-FHKR fusions in human and mouse. **IB, IC**	50 µl
Anti-Pax-5, Human (Rabbit)	PC700	A synthetic peptide corresponding to amino acid residues 1 - 15 of Pax-5 was used as an immunogen. Detects the ~50 kDa Pax-5 protein in human. **ELISA, IB**	100 µl
Anti-Sp1, Human (Rabbit)	PC701	A synthetic peptide corresponding to amino acid residues 520 - 534 of Sp1 was used as an immunogen. Detects the ~105 kDa Sp1 protein in human. **ELISA, IB**	100 µl
Anti-SSX, Human (Mouse)	OP196	Human GST-SSX2 fusion protein expressed in *E. coli* was used as an immunogen. Detects the ~29 kDa SSX2 protein in human. **IB, IF, IP, PS**	100 µl
Anti-Tal-1, Human (Rabbit)	PC702	A synthetic peptide corresponding to amino acid residues 7 - 21 of Tal-1 was used as an immunogen. Detects the ~45 kDa Tal-1 protein in K-562 cells. Reacts with human and mouse. **ELISA, IB**	100 µl
Anti-USF-1, Human (Rabbit)	PC703	A synthetic peptide corresponding to amino acid residues 1 - 14 of USF-1 was used as an immunogen. Detects the ~43 kDa USF-1 protein in human. **ELISA, IB**	100 µl
Anti-YY-1, Human (Rabbit)	PC704	A synthetic peptide corresponding to amino acid residues 109 - 123 of YY-1 was used as an immunogen. Detects the ~63 kDa YY-1 protein in human. **ELISA, IB**	100 µl

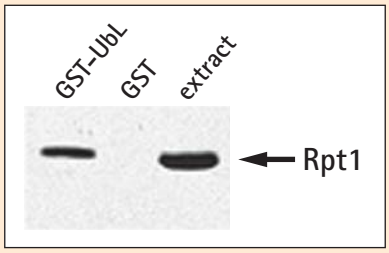
Key: ELISA: Enzyme-Linked Immunosorbent Assay; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation

Proteases and Proteasome in Protein Degradation

Eukaryotes possess two distinct protein degradation pathways, the endocytotic, lysosomal degradation pathway and the ATP-dependent, non-lysosomal degradation pathway. The non-lysosomal pathway degrades most intracellular proteins, including a variety of short-lived regulatory proteins such as cyclins, cyclin inhibitors, and oncogene products, and also the bulk of misfolded proteins. Protein degradation via this pathway is highly selective and involves covalent linking of proteins to multiple molecules of ubiquitin. Protein degradation by the Ubiquitin-ATP-Proteasome pathway is the most prolific and involves two distinct steps: (i) covalent attachment of ubiquitin to the target protein, which marks proteins for rapid degradation and (ii) degradation of the ubiquitin-protein conjugate by the 26S proteasome complex with the release of reusable ubiquitin.

Proteasome Isolation Kit, Human

- A rapid method for the isolation of biologically active proteasomes.
- Uses affinity matrix beads containing a ubiquitin-like domain (UbL) bound to GST-agarose.
- Proteasome bound to beads can be used in proteolytic assays using proteasome substrates.
- The proteasome subunit proteins can be identified by loading the beads directly onto SDS-PAGE gel and immunoblotting with subunit specific antibodies.
- Each kit contains UbL beads sufficient to process 12.5 to 25 mg of lysate, control glutathione-agarose beads, control lysate, and a set of instructions.



Cat. No. 539176 1 Kit

The first lane shows the recovery of the proteasome subunit Rpt1 with the proteasome-binding GST-UbL beads. The second lane is a mock-reaction (using GST), while the third lane contains 5% of the input lysate. The hydrolysis of Suc-LLVT-AMC is readily detected in the GST-UbL beads.

NEW! Antibodies for Protein Degradation Research

Product	Cat. No.	Comments	Size
Anti-CAND1, Human (Rabbit)	PC745	Detects ~120 kDa CAND1 that regulates the assembly of productive SCF ubiquitin ligases. The SCF ubiquitin E3 ligase complex controls the levels of several proteins involved in cell cycle control. Reacts with human, mouse, and rat. **IB, IF, IP**	100 µl
Anti-CHIP, Human (Rabbit)	PC711	Detects the ~35 kDa CHIP, a U-box dependent E3 ubiquitin ligase in a range of species. CHIP interacts with Hsc70 and Hsp90 regulating protein triage decisions between folding and degradation. **IB, IF, IP**	100 µl
Anti-CIN85, Internal, Human (Rabbit)	231005	Recognizes CIN85, a protein involved in the degradation of several tyrosine kinase receptors in human, mouse, porcine, and rat, including several growth factor receptors. CIN85 is an ubiquitously expressed adaptor protein with three SH3 domains. **IB, IF, IP**	100 µl
Anti-CIN85, C-Terminal, Human (Rabbit)	231006	Detects the ~85 kDa CIN85, a protein involved in the degradation of several tyrosine kinase receptors. Also detects the mono-ubiquinated form of CIN85. **ELISA, IB, IF, IP**	100 µl
Anti-Ubiquitin Conjugating Enzyme E2 N, Human (Rabbit)	662118	Immunogen used was a mixture of synthetic peptides corresponding to amino acid sequences 2 - 19 and 131 - 148 of UBE2 N. Detects the ~17 kDa UBE2 N protein in human. **IB**	100 µg

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **IB:** Immunoblotting; **IF:** Immunofluorescence; **IP:** Immunoprecipitation

NEW! Caspase Detection Kits

These kits employ a fast and convenient detection method based on the principle that the cell-permeable fluorescent markers irreversibly bind to the activated caspase in living cells. Caspases can then be detected by measuring the fluorescence intensity. Each kit is provided in a 100-test format and includes either FITC or RED substrate conjugated to FMK, wash buffer, unlabeled caspase inhibitor, and a directional insert.

Sample Type: Intact cells Assay Time: 1.5 Hours

Caspase-8 Detection Kit (FITC-IETD-FMK)

Fluorescent marker: FITC-IETD-FMK
(Ex. max.: ~485 nm; Em. max.: ~535 nm)

Inhibitor included: Z-VAD-FMK

Cat. No. QIA113 1 Kit

Caspase-9 Detection Kit (FITC-LEHD-FMK)

Fluorescent marker: FITC-LEHD-FMK
(Ex. max.: ~485 nm; Em. max.: ~535 nm)

Inhibitor included: Z-VAD-FMK

Cat. No. QIA115 1 Kit

Caspase-8 Detection Kit (Red-IETD-FMK)

Fluorescent marker: Red-IETD-FMK
(Ex. max.: ~540 nm; Em. max.: ~570 nm)

Inhibitor included: Z-VAD-FMK

Cat. No. QIA114 1 Kit

Caspase-9 Detection Kit (Red-LEHD-FMK)

Fluorescent marker: Red-LEHD-FMK
(Ex. max.: ~540 nm; Em. max.: ~570 nm)

Inhibitor included: Z-VAD-FMK

Cat. No. QIA116 1 Kit

Also Available...

Caspase-3 Activity Detection Kit (FITC-DEVD-FMK)

Fluorescent marker: FITC-DEVD-FMK
(Ex. max.: ~485 nm; Em. max.: ~535 nm)

Inhibitor included: Z-DEVD-FMK.

Cat. No. QIA91 1 Kit

Caspase Activity Detection Kit (FITC-VAD-FMK)

Fluorescent marker: FITC-VAD-FMK
(Ex. max.: ~485 nm; Em. max.: ~535 nm)

Inhibitor included: Z-VAD-FMK

Cat. No. QIA90 1 Kit

Caspase-3 Activity Detection Kit (Red-DEVD-FMK)

Fluorescent marker: Red-DEVD-FMK
(Ex. max.: ~540 nm; Em. max.: ~570 nm)

Inhibitor included: Z-DEVD-FMK

Cat. No. QIA93 1 Kit

Caspase Activity Detection Kit (Red-VAD-FMK)

Fluorescent marker: Red-VAD-FMK
(Ex. max.: ~540 nm; Em. max.: ~570 nm)

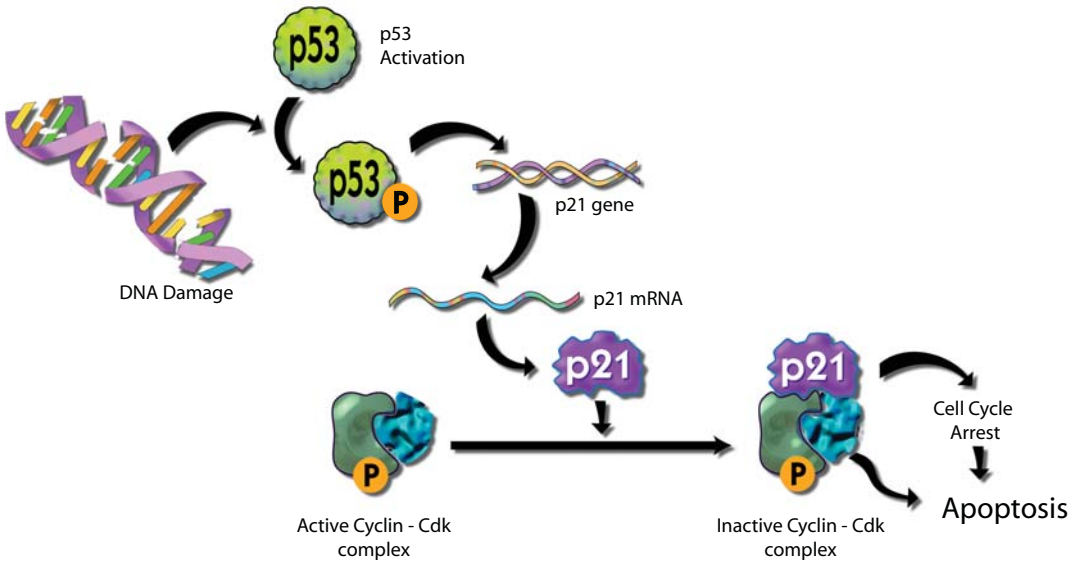
Inhibitor included: Z-VAD-FMK

Cat. No. QIA92 1 Kit

technical@apoptosis.com

Introducing ….. **NEW!** Antibodies for p53-Related Research

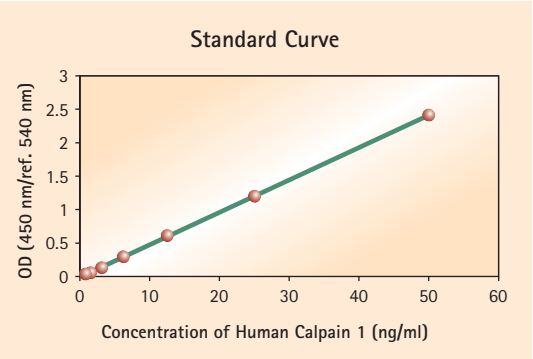
p53, a phosphoprotein of about 390 amino acids, can be activated by phosphorylation at many sites by stress-activated protein kinases, DNA protein kinase, casein kinases I and II, and cyclin-dependent kinases. When normal cells are subjected to stress signals, p53 is activated and its ubiquitin-dependent degradation is blocked. The resulting increase in p53 leads to the induction of apoptosis and/or cell cycle arrest. Cell cycle arrest allows DNA repair to proceed before replication or mitosis. In some cell types, however, p53 activation results in apoptosis as a means of eliminating irreparably damaged cells. Hence, functional p53 provides a protective effect against tumor growth. Mutations in the p53 gene are commonly seen in various types of human cancers. Any successful response to chemo- or radiation therapy is reduced in tumors containing mutant forms of p53.



Product	Cat. No.	Comments	Size
Anti-p53 Binding Protein 1 (Ab-1), Human (Rabbit)	PC712	Purified recombinant 53BP1 containing a His•Tag® sequence was used as an immunogen. Detects the ~230 kDa 53BP1, a protein which binds to the DNA-binding domain of p53 and enhances p53-mediated transcriptional activation. **IB, IF, IP**	100 µl
Anti-PUMA, Human (Rabbit)	PC686	A synthetic peptide corresponding to amino acid residues 2 – 16 was used as an immunogen. Detects the ~23 kDa PUMA, a BH3 domain-containing protein. Upon activation by p53, PUMA binds to Bcl-2, and localizes to the mitochondria to induce cytochrome c release and apoptosis. **IB**	100 µg
Anti-Peg3/Pw1, Mouse (Rabbit)	PC689	A protein fragment corresponding to amino acid residues 78 - 312 of Peg3/Pw1 expressed in *E. coli* was used as an immunogen. Peg3/Pw1 is up-regulated after DNA damage in a p53-dependent manner but has been reported to be induced in p53-mediated apoptosis, but not growth arrest. **IB, IF**	100 µl
Anti-HAUSP, Human (Rabbit)	DR1000	A synthetic peptide corresponding to amino acid residues 12 - 26 of human HAUSP was used as an immunogen. Detects the ~135 kDa HAUSP, a protein reported to stabilize p53 through the deubiquitination of p53. HAUSP has also been reported to be cleaved by caspase-3. **IB**	100 µg

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **IB:** Immunoblotting; **IF:** Immunofluorescence; **IP:** Immunoprecipitation

NEW! Calpain Assay Kits



Calpain 1 ELISA Kit

Sensitivity: ≤ 0.3 ng/ml Assay Time: 3.5 Hours

Sample Type: Plasma, serum, cell and tissue extracts

Provided with an antibody-coated microplate, calpain 1 standard, detector antibody, HRP-conjugated antibody, TMB substrate, assay diluent, CytoBuster™ protein extraction reagent, wash buffer concentrate, stop solution, plate sealers, and a directional insert.

Cat. No. QIA118 1 Kit

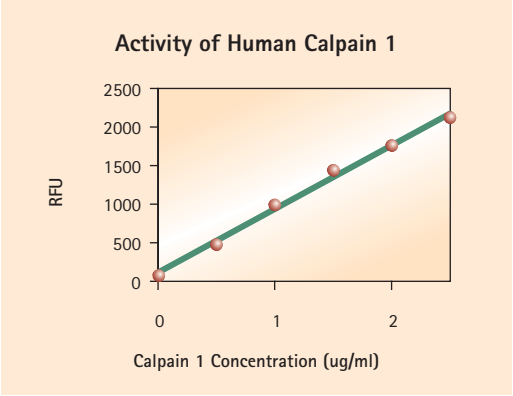
Calpain Activity Kit, Fluorogenic

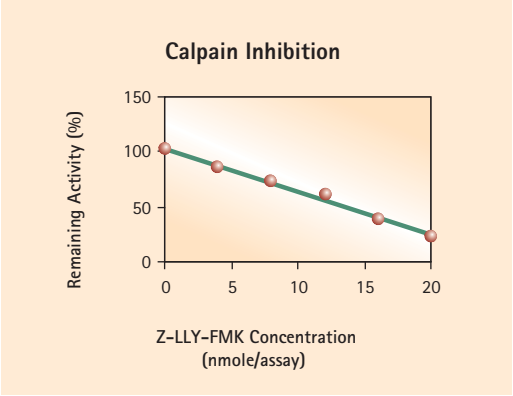
Assay Time: 1.5 Hours Sample Type: Cell lysates, plasma, and serum

Provided with a calibration standard, calpain 1 as positive control, substrate, activation buffer, inhibition buffer, reduction agent, assay buffer, cell lysis buffer, microtiter plate, plate sealer, and a directional insert.

Requires a fluorimeter or microplate reader (Ex. max.: ~360 nm; Em. max.: ~460 nm).

Cat. No. QIA120 1 Kit





Antibodies for Alzheimer's Disease Research

Anti-IDE, N-Terminal (97- 273), Rat (Rabbit)

A recombinant GST-fusion protein containing amino acid residues 97 - 273 of IDE was used as the immunogen.

Recognizes the ~115 kDa endogenous IDE as well as recombinant rat IDE.

IDE is a neutral, thiol-dependent, cytosolic zinc metalloprotease that is reported to break down β-amyloid. Suitable for ELISA, IB, and IC.

Cat. No. PC730 100 µl

Ref.: Morelli, L., et al. 2003. *J. Biol. Chem.* **278,** In Press; Kurochkin, I.V., and Goto, S., 1994. *FEBS Lett.* **345,** 33.

Anti-mAPH-1a^{L}, Human (Rabbit)

A synthetic peptide corresponding to amino acid residues 245 - 265 of human APH-1a^{L} was used as an immunogen.

Detects the ~30 kDa mAPH-1a^{L}, a mammalian homolog of the *C. elegans* anterior pharynx-defective-1 protein. mAPH-1 interacts with presenilin and nicastrin and is reported to be a functional component of the γ-secretase complex that is required for the intramembrane proteolysis of APP and Notch. Suitable for IB and IP.

Cat. No. PC728 **100 µl**

Ref.: Gu, Y., et al. 2003. *J. Biol. Chem.* **278,** 7374; Luo, W.J., et al. 2003. *J. Biol. Chem.* **278,** 7850; Lee, S.F, et al. 2002. *J. Biol. Chem.* **277,** 45013.

Anti-β-Amyloid (Asp-1) (FCA18), Human (Rabbit)

A synthetic peptide corresponding to the N-terminus of the β-amyloid peptide (Aβ) was used as immunogen.

Interacts only with the first free aspartyl- residue and recognizes the N-terminus portion of Ab1-x. Does not recognize aspartyl residues in full-length APP or N-acetylated aspartyl- or aspartyl-1 deleted Aβ peptides. Suitable for IB, IP, PS.

Cat. No. PC729 **25 µl**

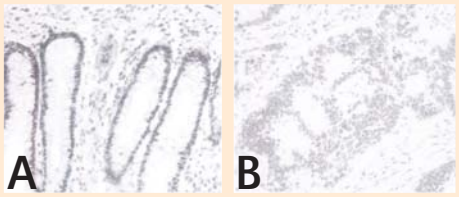
Ref.: Barelli, H., et al. 1997. *Mol. Med.* **3,** 695.

DNA Hypomethylation: A Risk Factor in Carcinogenesis

DNA methylation is a major determinant in the epigenetic silencing of genes. It is a complex process wherein three DNA methyltransferases catalyze the addition of a methyl group from S-adenosyl-L-methionine to the 5-carbon position of cytosine. It is involved in regulating transcription and chromatin structure, and protects cells from invading foreign DNA. DNA methylation occurs predominantly within the CpG dinucleotide and is one of the most prevalent epigenetic modifications of DNA in mammalian genomes. CpG sites are important spots for mutations in the germline and for inactivating mutations in tumor suppressor genes. About 25% of all mutations in the p53 gene in human cancers occur at CpG sites. The bulk of the genome in cancer cells becomes hypomethylated, in particular the normally hypermethylated and silent regions containing the repetitive elements are substantially demethylated. In a number of experimental models of carcinogenesis, this decrease in numbers of methyl groups appears to begin early in tumor progression.

References:

Clark, S.J., and Melki, J. 2002. *Oncogene* **21,** 5380; Robertson, K.D. 2002. *Oncogene* **21,** 5361; El-Osta, A., and Wolffe, A.P. 2000. *Gene Expr.* **9,** 63; Christman, J.K., et al. 1993. *Carcinogenesis* **14,** 551; Cooper, D.N., and Youssoufian, H. 1988. *Hum. Genet.* **78,** 151; Feinberg, A.P., et al. 1988. *Cancer Res.* **48,** 1159.



Indirect immunoperoxidase labeling of normal colon (A) and colon carcinoma (B) with Anti-5-methylcytosine (Mouse) (Cat. No. NA81).

Product	Cat. No.	Comments	Size
Anti-MBD1, Human (Mouse)	472519	Immunogen used was a synthetic peptide corresponding to a portion of MBD1. Detects the ~70 kDa MBD1 protein. MBD1 binds to sites of methylated DNA to repress transcription. **IB**	100 µg
Anti-MBD2, Human (Mouse)	472521	A recombinant MBD2 C-terminal fragment (amino acid residues 150 - 410) containing a His•Tag® sequence was used as an immunogen. MBD2 is an ~48 kDa protein which is reported to bind to methylated DNA and demethylate it. MBD2 may serve as a methylation dependent transcriptional repressor. **IB**	100 µg

Key: IB: Immunoblotting

DNA Hypomethylation, cont.

Product	Cat. No.	Comments	Size
Anti-MBD3, Human (Mouse)	472526	Detects the ~50 kDa MBD3 protein. MBD3 does not bind to methylated DNA. It has been reported that MBD3 is necessary for the physical interaction of histone deacetylase 1 and MTA2. MBD3 is also believed to serve as a substrate for Aurora A kinase. **IB**	100 µg
Anti-MBD4, Human (Rabbit)	472527	Two synthetic peptides corresponding to amino acid residues 268 - 282 and 337 - 352 of MBD4 were used as immunogen. Detects the ~64 kDa MBD4 protein which has been reported to suppress CpG mutability and tumorigenesis. **IB**	100 µg
Anti-5-Methylcytosine (Mouse)	NA81	5-Methylcytosine conjugated to ovalbumin was used as immunogen. Reacts with a broad range of species. **ELISA, FC, FS, IB, IF, PS, RIA**	50 µg

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **FS:** Frozen Sections; **IB:** Immunoblotting; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections; **RIA:** Radioimmunoassay

NEW! Antibodies for Angiogenesis and Wound Healing

Product	Cat. No.	Comments	Size
Anti-Angiopoietin-3, Mouse (Goat)	PC671	Detects the ~66 kDa mouse angiopoietin-3. **ELISA, IB, IH**	100 µg
Anti-Angiopoietin-Like Factor, Human (Goat)	PC672	Detects the ~45 kDa angiopoietin-like factor also known as cornea-derived transcript 6 (CDT6), a secreted protein expressed at high levels in the avascular corneal stromal layer. **ELISA, IB**	100 µg
Anti-Proepithelin, Human (Rabbit)	IM1000	Detects the ~67 kDa epithelial growth factor Proepithelin , a mediator of wound repair. Proepithelin has been reported to prevent neutrophil activation by TNF. Reacts with human and mouse. **IB, IP**	100 µg
Anti-Secretory Leukocyte Protease Inhibitor, Mouse (Rabbit)	IM1001	Detects the ~12 kDa Secretory Leukocyte Protease Inhibitor, a serine proteinase inhibitor which plays a role in wound healing by preventing the cleavage of proepithelin by elastase. Reacts with human and mouse. **IB, IP**	100 µg
Anti-VEGF Receptor 2, Mouse (Mouse)	676486	Detects the ~230 kDa VEGFR2 protein in human and mouse. Also detects an immature form at ~200 kDa. **IB**	50 µg
Anti-VEGF Receptor 2, Mouse (Rabbit)	676488	Detects the ~230 kDa VEGFR2 protein in human and mouse and also detects an immature form of the protein at ~200 kDa. May also detect degradation products at 97 and 70 kDa in lysates in the absence of APMSF. **IB, IP**	50 µl

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **IB:** Immunoblotting; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

technical@apoptosis.com

Kits for Angiogenesis and Tumor Metastasis Research

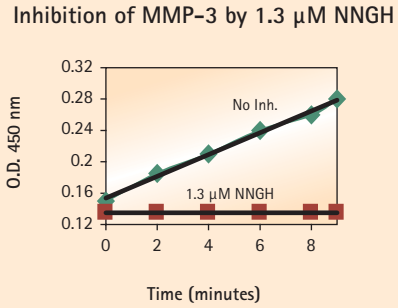
MMP-3 Inhibitor Screening Assay Kit, Colorimetric

Sample Type: Non-Thiol containing natural or synthetic inhibitors

Kit Contains: Recombinant human MMP-3, MMP colorimetric substrate, control MMP-3 inhibitor, assay buffer, microtiter plate, and a directional insert.

Comments: A thiopeptolide colorimetric substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LGOC$_2$H$_5$), is used to measure the activity of MMP-3 in the presence or absence of potential inhibitors. Thiol inhibitors should not be tested with this kit.

Cat. No. QIA103 **1 Kit**



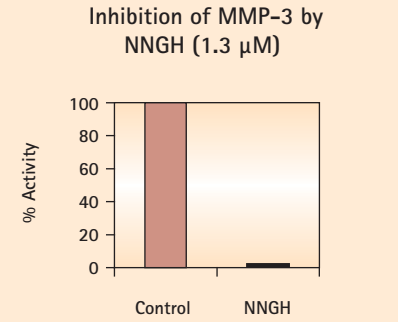
MMP-3 Inhibitor Screening Assay Kit, Fluorometric

Sample Type: Natural or synthetic inhibitors

Kit Contains: Recombinant human MMP-3 inhibitor, MMP fluorogenic substrate, calibration standard, control MMP-3 inhibitor, assay buffer, microtiter plate, and a directional insert.

Comments: A fluorescent substrate (MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH$_2$) is used to measure the activity of MMP-3 in the presence or absence of potential inhibitors.

Cat. No. QIA104 **1 Kit**



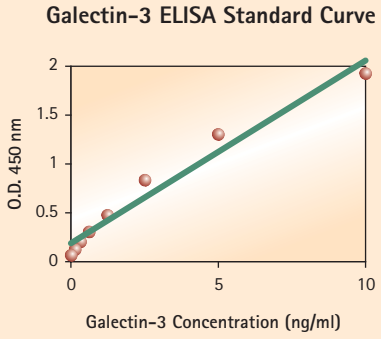
NEW! Galectin-3 ELISA Kit

Format: 96 tests Assay Range: 0.16 - 10 ng/ml

Assay Time: ~4 hours

Sample Type: Cell culture supernatants, plasma, serum, or other biological fluids.

Kit Contains: Coated microplate, anti-galectin-3 (Rabbit), anti-rabbit-HRP, galectin-3 standard (2 vials), assay buffer concentrate, wash buffer concentrate, sample diluent, substrate solution I, substrate solution II, stop solution, blue-dye, red-dye, green-dye, plate covers, and a detailed insert.



Comments: Galectin-3 is a 31 kDa β-galactoside-binding protein that is involved in cell growth, adhesion, and differentiation, and in tumor progression. Galectin-3 has been reported to inhibit apoptosis. Expression of galectin-3 has been shown to have a strong correlation with the grade and malignant potential of primary brain tumors.

Cat. No. QIA112 **1 Kit**

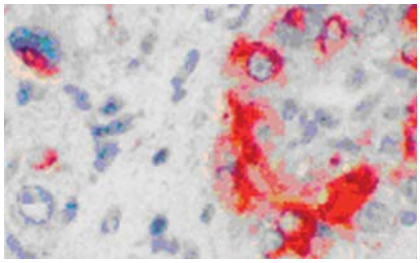
NEW! Antibodies for Breast Cancer Markers

Anti-BRST-1, Human (Mouse)

BCA-225 protein secreted from the T47D human breast carcinoma cell line was used as an immunogen. Shows strong intracytoplasmic staining in primary and metastatic breast carcinoma tissue and in cervical carcinomas.

Cat. No. CA1000 **200 µl**

Ref. Tsubura, A., et al. 1992. *J. Cutan. Pathol.* **19,** 73; Hayes, D.F., et al. 1991. *J. Clin. Oncol.* **9,** 1113.

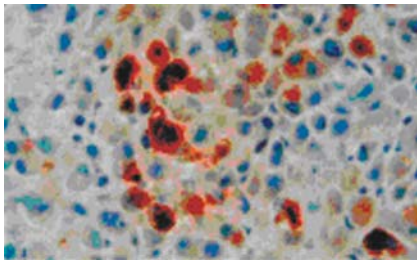


Cytoplasmic staining in breast carcinoma tissue.

Anti-BRST-2, Human (Mouse)

Gross cystic disease fluid protein-15 was used as an immunogen. Detects Gross Cystic Disease Fluid Protein 15 (GCDFP-15), expressed in the metaplastic epithelium of breast tissue. GCDFP-15 is a useful marker for the detection of breast carcinoma and the identification of metastasis of breast origin.

Cat. No. CA1001 **200 µl**

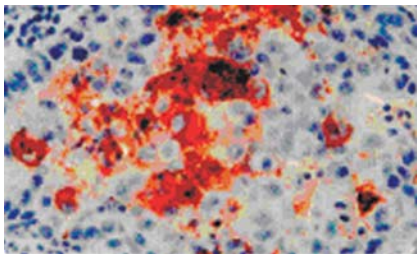


Staining seen in lobular breast carcinoma.

Anti-BRST-3, Human (Mouse)

A membrane-enriched fraction of a breast carcinoma metastatic to the liver was used as an immunogen. Detects TAG-72, a 220-440 kDa tumor-associated oncofetal protein complex expressed in a wide variety of adenocarcinomas including invasive ductal breast carcinoma, colon, pancreatic, and gastric cancers.

Cat. No. CA1002 **200 µl**



BRST-3 staining seen in breast carcinoma.

Antibodies to Steroid Receptor Co-Activators

Product	Cat. No.	Comments	Size
Anti-SRC-2, Mouse (Rabbit)	567821	A recombinant protein containing amino acids 787 - 1100 of SRC-2 and a His•Tag® sequence was used as an immunogen. Detects the ~180 kDa SRC-2. Members of the SRC family have been reported to enhance the transcriptional activities of several nuclear hormone receptors in response to their respective ligands. Does not cross-react with other SRC family members. **IB, IF, IP**	100 µl
Anti-SRC-3, Mouse (Rabbit)	567822	A recombinant protein containing amino acids 590 - 851 of SRC-3 and a His•Tag® sequence was used as an immunogen. Detects the ~180 kDa SRC-3, a nuclear receptor co-activator which interacts with nuclear receptors to enhance their trans-activation in a ligand dependent manner. SRC-3 has been reported to contain histone acetyltransferase activity and to interact with NF-κB to enhance its transcriptional activity. **IB, IF, IP, PS**	100 µl

Key: IB: Immunoblotting; **IF:** Immunofluorescence; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

Prices and availability are subject to change without notification.

web: www.apoptosis.com

e-mail: technical@apoptosis.com

or contact your local sales office