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The Ubiquitin-Proteasome Pathway:

A Link with NF-κB Activation

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The Ubiquitin-Proteasome Pathway:

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PRODUCTS

Ubiquitin-Proteasome Pathway:

Complex Nature of Proteasome Complex

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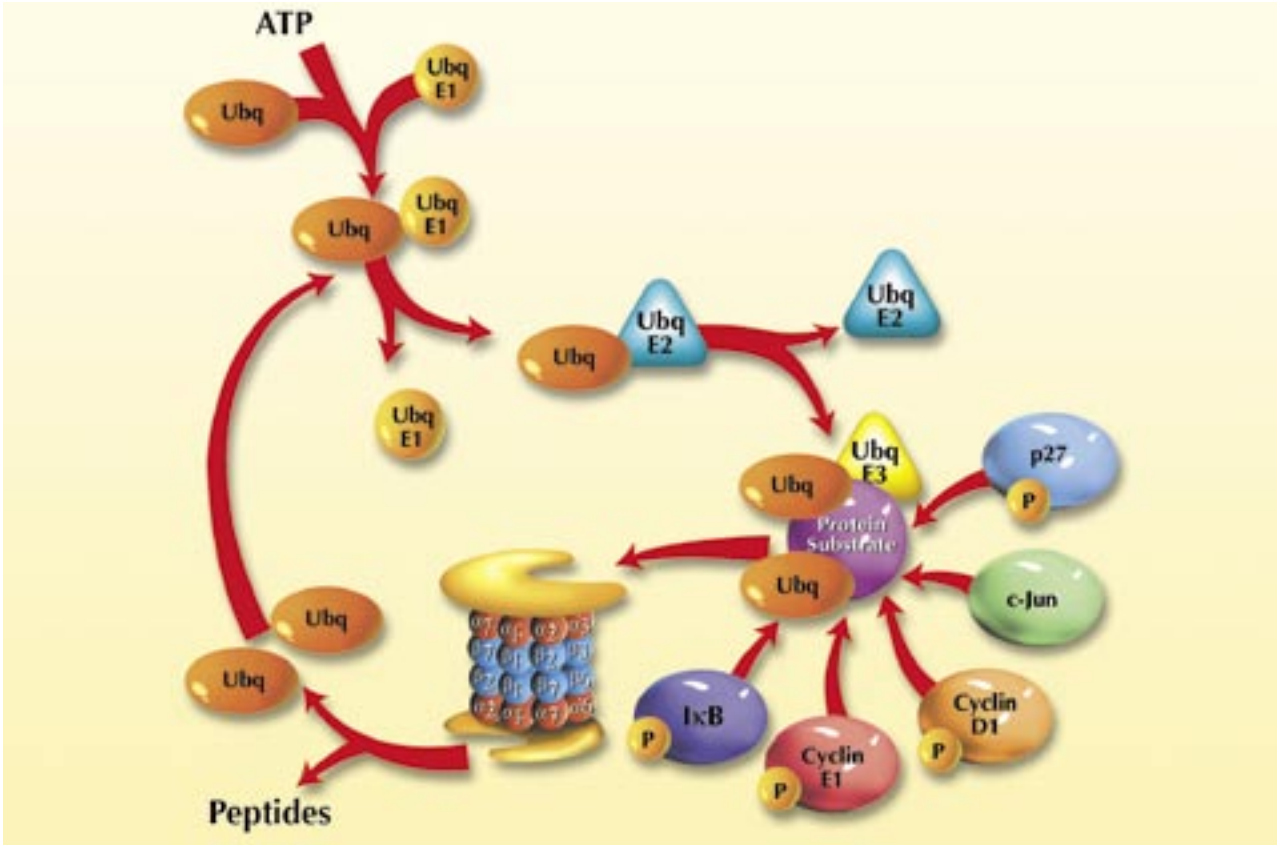
Merck Biosciences

Proteasomes are large multi-subunit protease complexes that are localized in the nucleus and cytosol and selectively degrade intracellular proteins. They play a major role in the degradation of proteins involved in cell cycling, proliferation, and apoptosis and a vast majority of short-lived proteins are degraded by the ubiquitin-proteasome pathway. A protein marked for degradation is covalently attached to multiple molecules of ubiquitin (Ub), a highly conserved 76-amino acid (8.6 kDa) protein, which escorts it for rapid hydrolysis to the multi-component enzymatic complex known as the 26S proteasome. The functionally active 26S proteasome is 2.4 mDa complex and the proteolytic core of this complex, the 20S proteasome, contains multiple peptidase activities and functions as the catalytic machine. This core is composed of 28 subunits arranged in four heptameric, tightly stacked rings (α7, β7, β7, α7) to form a cylindrical structure. The α-subunits make up the two outer and the β-subunits the two inner rings of the stack. The entrance of substrate proteins to the active site of the complex is guarded by the α-subunits that allow access only to unfolded and extended polypeptides. The proteolytic activity is confined to the β-subunits.

The regulatory unit of the 26S proteasome is known as the 19S (PA 700) particle, which consists of at least 20 polypeptide subunits that include ATPases, a de-ubiquitinating enzyme, and polyubiquitin-binding subunits. ATPases function to continuously supply energy for selective degradation of target proteins. Energy is required for unfolding of proteins to allow them to penetrate the channel of α- and β-rings of the 20S proteasome. Mammalian cells also contain another regulatory complex that associates with the 20S proteasome. It is referred to as the 11S regulator of PA28. PA28 is a ring-shaped particle that associates with the 19S unit at both ends of the 20S proteasome and functions as an activator protein. It is composed of two homologous proteins, PA28α and PA28β, and is reported to stimulate peptidase activities without affecting the degradation of large protein substrates.

The proteasome identifies proteins that have been targeted for degradation by their Ub tags. In the ubiquitin-proteasome degradation pathway, ubiquitin is first covalently ligated at a lysine residue of the target protein by a multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and the Ub-ligating



Ubiquitin Proteasome Pathway

(E3) enzymes, which act in a sequential manner. In the initiation step E1 activates an Ub monomer at its C-terminal cysteine residue to a high-energy thiolester bond, which is then transferred to a reactive cysteine residue of the E2 enzyme. Over 25 different types of E2 have been described in mammalian cells. The final transfer of ubiquitin to the ε-amino group of a reactive lysine residue of substrate proteins is brought about by the E3, the Ub-ligase enzyme. In mammalian cells hundreds of E3 have been described, each binding to a specific protein substrate that has been targeted for proteasomal degradation. Ubiquitinated protein is escorted to the proteasome where it undergoes final degradation and the ubiquitin is released and recycled. The unique and distinguishing feature of the proteasome is the presence of multiple peptidase activities that include chymotrypsin-like (cleavage after hydrophobic side chains), postglutamyl peptidase (cleavage after acidic side chains), and trypsin-like (cleavage after basic side chains) activities. It has been proposed that the intact protein substrates are first recognized by the 19S unit, which allows them to enter the proteasome cavity where PA28 stimulates their cleavage by peptidases.

The ubiquitin-proteasome pathway plays a major role in the breakdown of abnormal proteins that result from oxidative damage and mutations. The reactive oxygen species can promote partial unfolding of the proteins, exposing hydrophobic domains to proteolytic enzymes of the 20S complex. Rapid degradation of defective enzymes, as seen in diseases caused by metabolic abnormalities, also occurs in the proteasome. However, it is not known how the Ub system recognizes the abnormal state of these proteins.

The Ub-proteasome pathway has been implicated in several forms of malignancy, in the pathogenesis of several genetic diseases, and in diseases associated with muscle wasting. It is also involved in the destruction of proteins that participate in cell cycle progression, transcription control, signal transduction, and metabolic regulation. Degradation of Cdk activators and inhibitors by the proteasome complex regulates the progression of the cell cycle. It is believed that phosphorylation of various proteins, such as cyclin E, cyclin D, p27, IκBα, and STAT1 allows them to be ubiquitinated and marked for proteolysis in the proteasome complex. On the other

hand, phosphorylation of certain other proteins, such as c-Fos and c-Jun, prevents their ubiquitination and proteasomal degradation. This further indicates a direct involvement of the proteasome in cell proliferation and the cell cycling processes. Several elements of the cell cycling process that are degraded in the Ub-proteasome pathway are potential targets for deregulation in cancer. For example, cyclins B, D1, and E are rapidly degraded in the proteasome and are overexpressed in breast tumor cell lines. Alterations in proteasome activity in tumors have also been linked to multi-drug resistance. The study of Ub-dependent degradation of p53, a tumor suppressor gene product, has opened up a new arena of research in apoptosis and cancer. The accumulation of p53 is thought to occur mainly via the down-regulation of its degradation in the proteasome. Human papilloma virus (HPV)-related cancers are linked to an upregulation of p53 degradation in the Ub-proteasome pathway. Low levels of p27, an inhibitor of the Cdk complex reported in several common tumors, are shown to be due to its increased degradation in the proteasome complex. Another area currently under investigation is the mechanism by which nuclear de-ubiquitinating enzyme, BAP1, binds to the breast cancer suppressor, BRCA1. Augmentation of the growth suppressive effects of BRCA1 are attributed to the overexpression of BAP1.

As the dominant protease system dedicated to protein turnover, proteasomes play a vital role in shaping the protein repertoire in the cell. Several distinct groups of compounds, designed to act as proteasome inhibitors, have helped immensely in understanding the biological role and importance of the ubiquitin-proteasome pathway. These compounds block proteasome function without affecting other normal biological processes in the cell. The most notable of these is lactacystin, which acts as a covalent inhibitor of the chymotrypsin-like and trypsin-like activities of proteasome. This inhibitory effect is thought to be due to the action of its β-lactone form that is produced upon incubation in aqueous medium. Lactacystin is also shown to inhibit cell cycle progression and induce apoptosis in human monoblast cells. The vital nature of proteasomes in several cellular functions makes it a difficult, yet important, target in cancer chemotherapy. It has been shown that actively proliferating cancer cells are more susceptible to the action of proteasome inhibitors than non-cancerous cells. Lactacystin has been shown to induce apoptosis in human chronic lymphocytic leukemia cells, but not in normal lymphocytes. MG-132, another potent proteasome inhibitor, induces apoptosis in acute myelogenous leukemia (AML) stem cells, but does not affect normal $CD34^+$ stem cells at similar doses. Proteasome inhibitors have also been used to block the activation of the NF-κB pathway. Constitutively active NF-κB pathway is common in several solid tumors and proteasome inhibitors block this activation and make cancer cells more susceptible to radiation therapy and chemotherapeutic agents.

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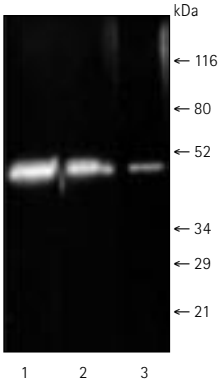
Assay Kits for Proteasome/Ubiquitin Research

Product	Cat. No.	Comments	Size
20S Proteasome Assay Kit, SDS-Activated	**539158**	Useful in quantifying 20S proteasome activity and for screening inhibitors. 20S activity is measured by monitoring the release of free AMC from the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC.	1 kit
26S Proteasome Degradation Kit	**539159**	Useful for measuring the degradation of pre-formed ubiquitin-protein conjugates by the 26S proteasome. Contains a partially purified protein fraction from rabbit cells containing 26S proteasome. ATP solution and a user protocol are provided. Does not contain the conjugation enzymes (E1, E2, or E3).	1 kit
Proteasome Isolation Kit, Human	**539176**	Useful for rapid isolation of biologically active proteasomes using affinity matrix beads comprised of a GST-fusion protein containing an ubiquitin-like domain (UbL) bound to glutathione-agarose. The proteasome subunit proteins can be identified by loading the beads directly onto an SDS-PAGE gel and immunoblotting with subunit-specific antibodies. Alternatively, proteasome bound to beads can be used in functional assays.	1 kit
Ubiquitin Protein Conjugation Kit	**662096**	Useful for the formation of ubiquitinated proteins that are labeled or are immuno-detectable. Has been shown to work with typical iodinated substrate proteins such as lysozyme and β-lactoglobulin. Addition of Ubiquitin Aldehyde (Cat. No. 662056) is recommended for the inhibition of ubiquitin C-terminal hydrolase and to improve conjugate yield. Fractions do not contain 20S, 26S, or any other conjugate or protein degradation activity. Each kit includes two enzyme fractions, ubiquitin, ATP solution, and a user protocol.	1 kit
Ubiquitinated Protein Enrichment Kit	**662200**	Useful for rapid isolation of ubiquitinated proteins using affinity beads comprised of a GST-fusion protein containing an ubiquitin-associated sequence bound to glutathione-agarose. Also useful for the enrichment of polyubiquitinated proteins from cell lysates and tissue homogenate can be used with a broad range of species including canine, human, mouse, and yeast. The ubiquitinated proteins can be identified by loading the beads directly onto an SDS-PAGE gel and then immunoblotting with the antibody of choice or Anti-Ubiquitin (Cat. No. 662099). Alternatively, beads can be treated with Isopeptidase T (Cat. No. 419700) to release the proteins from the ubiquitin chains.	1 kit

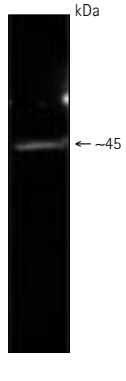
Antibodies for Proteasome/Ubiquitination Research

Product	Cat. No.	Comments	Applications	Size
Anti-19S Proteasome S2-Subunit Rabbit pAb	**539166**	Polyclonal IgG, purified. Detects the ~100 kDa human protein in HeLa cell extracts and partially purified 26S proteasome. A band at 50 kDa may also be detected corresponding to α subunit degradation product.	IB, IP	100 µl
Anti-19S Proteasome, S6-Subunit Rabbit pAb	**539169**	Polyclonal IgG, purified. Recognizes the ~48 kDa human protein in HeLa cell extracts and partially purified 19S proteasome preparations.	IB, IP	100 µl
Anti-19S Regulator ATPase Subunit Rpt1 Mouse mAb	**ST1058**	Monoclonal IgG_1, partially purified. Detects the ~48 kDa 19S Regulator ATPase Subunit Rpt1 protein, involved in the unfolding and translocation of substrates to the 20S proteasome's catalytic chamber. This antibody is not suitable for use in immunoprecipitation.	IB	100 µl
Anti-19S Regulator ATPase subunit Rpt4 Mouse mAb	**ST1059**	Monoclonal IgG_{2a}, purified. Detects the ~44 kDa 19S regulator ATPase subunit Rpt4 protein, involved in the unfolding and translocation of substrates in the 20S proteasome's catalytic chamber. This antibody is not suitable for use in immunoprecipitation.	IB	100 µl
Anti-19S Regulator non-ATPase Subunit Rpn10 Mouse mAb	**ST1060**	Monoclonal IgG, purified. Detects the ~45 kDa 19S regulator non-ATPase subunit Rpn10 protein, a non-ATPase subunit of the 19S regulatory complex of the 26S proteasome.	IB, IP	100 µl
Anti-20S Proteasome α-Subunit *Methanosarcina thermophila* Rabbit pAb	**539153**	Polyclonal IgG, undiluted serum. Does not cross-react with the β-subunit. Shows cross-reactivity with most mammalian species.	IB	100 µl
Anti-20S Proteasome α1-Subunit Rabbit pAb	**539145**	Polyclonal IgG, affinity purified. Detects the ~29 kDa protein in cell tissue lysates and tissue homogenate. Reacts with most mammalian species.	IB	100 µg
Anti-20S Proteasome α1, 2, 3, 5, 6, & 7-Subunits Mouse mAb	**ST1049**	Monoclonal IgG_1, purified. Reacts with six different α-type subunits. In ELISA the antibody reacts with the peptide sequence TVWSPQGRLHQVEYAMEA encompassing the prosbox I motif common to α-type.	ELISA, IB	100 µl
Anti-20S Proteasome α3-Subunit Mouse mAb	**ST1050**	Monoclonal IgG_1, purified. Detects the ~30 kDa 20S proteasome α3-subunit protein. The 20S proteasome α3-subunit (HC9) has been identified as a major target of the humoral autoimmune response in patients with autoimmune myositis and systemic lupus erythematosus.	IB, PS	100 µl
Anti-20S Proteasome α5-Subunit Mouse mAb	**ST1051**	Monoclonal IgG_{2a}, purified. Detects the ~28 kDa 20S proteasome α5-subunit protein, involved in an ATP/ubiquitin-dependent non-lysosomal proteolytic pathway. Does not immunoprecipitate HeLa cell preparation, but does precipitate raw cell extracts.	IB, IP	100 µl

ELISA = enzyme-linked immunosorbent assay • FS = frozen section • GS = gel shift assays • IB = immunoblotting
IC = immunocytochemistry • IH = immunohistochemistry • IP = immunoprecipitation • PS = paraffin sections



Detection of human 19S regulator ATPase subunit Rpt1 by immunoblotting. Samples: Whole cell lysates from HeLa cells (lane 1), purified 26S proteasome (lane 2), and human placental proteasome (lane 3). Primary antibody: Anti-19S Regulator ATPase Subunit Rpt1 Mouse mAb (MSS1-04) (Cat. No. ST1058) (1:5000).



Detection of human 19S regulator ATPase subunit Rpn10 by immunoblotting. Sample: Whole cell lysate from HeLa S3 S100 cells. Primary antibody: Anti-19S Regulator non-ATPase Subunit Rpn10 Mouse mAb (S5a-18) (Cat. No. ST1060) (1:1000).

Antibodies for Proteasome/Ubiquitination Research *continued*

Product	Cat. No.	Comments	Applications	Size
Anti-20S Proteasome α7-Subunit Mouse mAb	**ST1052**	Monoclonal IgG_1, partially purified. Detects the ~30 kDa 20S proteasome α7-subunit protein, which is involved in an ATP/ubiquitin-dependent non-lysosomal proteolytic pathway. The α7-subunit has been reported to interact with aurora B.	IB, IP, PS	100 µl
Anti-20S Proteasome β-Subunit Rabbit pAb	**539156**	Polyclonal IgG, partially purified. Does not cross-react with the α-subunit. Cross-reacts with most mammalian species.	IB	100 µl
Anti-20S Proteasome β1-Subunit Mouse mAb	**ST1054**	Monoclonal IgG_1, partially purified. Detects the ~29 kDa 20S proteasome β1-subunit protein, which is involved the ATP/ubiquitin-dependent non-lysosomal proteolytic pathway.	IB	100 µl
Anti-20S Proteasome β3-Subunit Mouse mAb	**ST1055**	Monoclonal IgG_1, partially purified. Detects the ~23 kDa 20S proteasome β3-subunit protein, involved in the ATP/ubiquitin-dependent non-lysosomal proteolytic pathway. Does not immunoprecipitate intact proteasomes from HeLa cell preparations.	IB	100 µl
Anti-20S Proteasome β4-Subunit Rabbit pAb	**ST1056**	Polyclonal IgG, partially purified. Detects the ~23 kDa 20S proteasome β4-subunit protein, involved in the ATP/ubiquitin-dependent non-lysosomal proteolytic pathway.	IB	100 µl
Anti-20S Proteasome β5i-Subunit Rabbit pAb	**ST1057**	Polyclonal IgG, partially purified. Detects the ~26 kDa 20S proteasome subunit β5i, a subunit of the immunoproteasome. Immunoproteasome subunits are stimulated by γ-interferon and substitute for standard proteasome subunits. May detect an additional band at ~30 kDa.	IB, PS	100 µl
Anti-20S Proteasome Core Subunits Rabbit pAb	**ST1053**	Polyclonal IgG, partially purified. Detects ~25-30 kDa 20S proteasome core subunits (α5, α7, β1, β5i, and β7).	IB, IP, PS	100 µl
Anti-26S Proteasome, S4-Subunit Yeast (*Saccharomyces pombe*) Rabbit pAb	**539167**	Polyclonal IgG, purified. Recognizes the ~56 kDa protein in HeLa cell extracts and partially purified human 26S proteasome. A lesser band of ~35 kDa may also be detected corresponding to a subunit degradation product.	IB, IP	100 µl
Anti-26S Proteasome, S5A-Subunit Rabbit pAb	**539168**	Polyclonal IgG, purified. Recognizes the ~50 kDa human protein in HeLa cell extracts and partially purified 26S proteasome preparations.	IB, IP	200 µl
Anti-26S Proteasome, S6'-Subunit Rabbit pAb	**539170**	Polyclonal IgG, purified. Recognizes the ~48 kDa human protein in HeLa cell extracts and partially purified 26S proteasome preparations.	IB, IP	100 µl
Anti-26S Proteasome, S7-Subunit Rabbit pAb	**539171**	Polyclonal IgG, purified. Recognizes the ~47 kDa human protein in HeLa cell extracts and partially purified 26S proteasome preparations.	IB, IP	100 µl

ELISA = enzyme-linked immunosorbent assay • FS = frozen section • GS = gel shift assays • IB = immunoblotting
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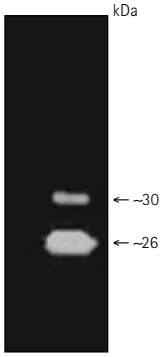
Detection of human 20S proteasome β4-subunit by immunoblotting.
Samples: Purified human erythrocyte-derived 20S proteasome (lanes a, b) and HeLa S3 S100 cytosolic preparation (lanes c, d). Primary antibody: Anti-20S proteasome β4 subunit Rabbit pAb (Cat. No. ST1056) (1:1000) without blocking peptide (lanes a, c) or with blocking peptide (lanes b, d).



Detection of human 20S proteasome β5i-subunit by immunoblotting.
Samples: Whole cell lysates from human lymphoblastic B cell lines untransfected (LMP7) (lane a) or double transfected with cDNA encoding β5i LMP7 or LMP2 (lane b). Primary antibody: Anti-20S Proteasome β5i-Subunit Rabbit pAb (Cat. No. ST1057) (1:20,000).

Antibodies for Proteasome/Ubiquitination Research *continued*

Product	Cat. No.	Comments	Applications	Size
Anti-26S Proteasome, S8-Subunit Rabbit pAb	**539172**	Polyclonal IgG, purified. Recognizes the ~45 kDa human protein in HeLa cell extracts and partially purified 26S proteasome preparations.	IB, IP	100 µl
Anti-26S Proteasome, S10B-Subunit Rabbit pAb	**539173**	Polyclonal IgG, purified. Recognizes the ~42 kDa human protein in HeLa cell extracts and partially purified 26S proteasome preparations. A band at ~30 kDa may also be detected corresponding to a subunit degradation product.	IB, IP	100 µl
Anti-CSN3 Rabbit pAb	**ST1043**	Polyclonal IgG, immunoaffinity-purified. Detects the ~40 kDa CSN3, a subunit of the COP9 signalosome complex.	IB	100 µl
Anti-CSN4 Rabbit pAb	**ST1044**	Polyclonal IgG, immunoaffinity-purified. Detects the ~40 kDa CSN4, a subunit of the COP9 signalosome complex.	IB	100 µl
Anti-Hip-2 Rabbit pAb	**NE1011**	Polyclonal IgG, undiluted serum. Detects the ~25 kDa E2-25K/Hip2, an ubiquitin conjugating enzyme that has been reported to play a role in mediating amyloid-β neurotoxicity.	FS, IB, IP, PS	50 µl
Anti-Jab1/CSN5 Rabbit pAb	**ST1045**	Polyclonal IgG, immunoaffinity-purified. Detects the ~33 kDa Jab1/CSN5, a subunit of the COP9 signalosome complex.	IB	100 µl
Anti-PGP9.5 Rabbit pAb	**NE1013**	Polyclonal IgG, undiluted serum. Detects the ~26 kDa PGP9.5, a ubiquitin hydrolase widely expressed in neuronal tissues and overexpressed in some cancers.	IB, PS	50 µl
Anti-Proteasome Activator PA28α Subunit, Rabbit pAb	**539146**	Polyclonal IgG, affinity purified. Recognizes the ~28 kDa protein in canine, hamster, human, mouse, and rat cell and tissue lysates.	IB	100 µg
Anti-Proteasome Activator PA28γ Subunit (1-14) Rabbit pAb	**539148**	Polyclonal IgG, purified. Recognizes the ~28 kDa γ-subunit of PA28 in cell and tissue lysates.	IB	100 µg
Anti-Proteasome Activator PA700 Subunit 10B Rabbit pAb	**539147**	Polyclonal IgG, affinity purified. Recognizes the ~42 kDa subunit 10B, as well as an ~46 kDa band most likely corresponding to the combined signal of subunits 6B, 7, and 8, in cell and tissue lysates. Also recognizes to a lesser extent bands at 53, 75, 84, and 90 kDa.	IB	100 µg
Anti-STAM1 Rabbit pAb	**ST1040**	Polyclonal IgG, immunoaffinity-purified. Detects the ~68 kDa STAM1 in human and mouse. STAM1 is a cytoplasmic adaptor protein that plays a major role in the sorting of ubiquitinated proteins.	IB, IP	50 µg
Anti-STAM2 Rabbit pAb	**ST1038**	Polyclonal IgG, immunoaffinity-purified. Detects the ~58 kDa STAM2 in human and mouse. STAM2 plays a major role in the sorting of ubiquitinated proteins.	IB, IP	50 µg
Anti-Ubc9 Rabbit pAb	**PC131**	Polyclonal IgG, purified. Recognizes the 18 kDa Ubc9 protein in human and mouse. In some cell lines an unrelated protein with a molecular weight of ~50 kDa is detected by immunoblotting.	IB	100 µg
Anti-Ubiquitin Rabbit pAb	**662099**	Polyclonal IgG, undiluted serum. Recognizes free and conjugated ubiquitin. Ubiquitin is a 76-amino acid protein that attaches to proteins in a multimeric chain.	IB, IH, IP	50 µl
Anti-Ubiquitin (Ab-1) Mouse mAb (6C1)	**CC37**	Monoclonal IgG_{2a}, undiluted ascites. Detects a ~9.6 kDa band of recombinant ubiquitin and a broad range of ubiquitinated proteins in lactacystin-treated cells.	IB	100 µl
Anti-Ubiquitin-Activating Enzyme E1A Rabbit pAb	**662102**	Polyclonal IgG, undiluted serum. Reacts with human E1A. Does not cross-react with other proteins by immunoblotting.	IB, IH	200 µl
Anti-Ubiquitin-Activating Enzyme E1A/E1B Rabbit pAb	**662106**	Polyclonal IgG, undiluted serum. Reacts with human E1A and E1B. Does not cross-react with other proteins by immunoblotting.	IH	200 µl

ELISA = enzyme-linked immunosorbent assay • FS = frozen section • GS = gel shift assays • IB = immunoblotting
IC = immunocytochemistry • IH = immunohistochemistry • IP = immunoprecipitation • PS = paraffin sections

Ubiquitination Enzymes and Related Products

Product	Cat. No.	Comments	Size
NEW ISG15-Activating Enzyme E1, Human	**662076**	A ubiquitin-like protein (UBI) that consist of two Ub (ubiquitin)-related domains, N-terminal and C- terminal domains identical to UBIs conjugated to cellular proteins after IFNα/β stimulation. ISG15-activating enzyme is responsible for the first step in ISG15-protein isopeptide bond formation and is a critical component for the initiation of any *in vitro* conjugation reaction. *Purity: ≥98% by SDS-PAGE.*	25 µg
Fraction I, HeLa S3 Cell	**344250**	Protein fraction from HeLa S3 cells that does not bind to anion exchange resins. Contains E2s, E3s, and ubiquitin. Useful for testing the requirement of Fraction I enzymes for the degradation of protein substrates *in vitro*, and for measurement of activity of protein conjugates *in vitro*. May require Fraction II (Cat. No. 344255) for activity when used with proteasome inhibitors and Ubiquitin Aldehyde (Cat. No. 662056).	1 mg
Fraction II, HeLa S3 Cells	**344255**	Protein fraction from HeLa S3 cells that binds to anion exchange resins. Contains E1, E2s, E3s, PA28, 20S and 26S proteasomes, and ubiquitin C-terminal hydrolases (UCHs). Useful for demonstrating ubiquitin and/or ATP-dependent degradation of protein substrates *in vitro*, as well as for measurement of activity of protein conjugates *in vitro* in the presence of proteasome inhibitors and Ubiquitin Aldehyde (Cat. No. 662056).	1 mg
Fraction II, Rabbit Reticulocytes	**344260**	Protein fraction from rabbit reticulocytes that binds to anion exchange resins. Contains E1, E2s, E3s, PA28, 20S and 26S proteasomes, and ubiquitin C-terminal hydrolases (UCHs). Useful for demonstrating ubiquitin and/or ATP-dependent degradation of protein substrates *in vitro*, as well as for measurement of activity of protein conjugates *in vitro* in the presence of proteasome inhibitors and Ubiquitin Aldehyde (Cat. No. 662056).	2.5 mg
Isopeptidase T, Rabbit	**419700**	A member of the higher molecular weight group of ubiquitin C-terminal hydrolases (UCHs) involved in the hydrolysis of isopeptide linkages of polyUb chains. Isopeptidase T activity is regulated by ubiquitin levels. Plays a major role in ubiquitin recycling and in the regulation of 26S proteasome activity.	25 µg
NEW SUMO-Activating Enzyme E1 (Aos1/Uba2), GST-Fusion, Human	**662073**	SUMO (small ubiquitin-related modifier)-activating enzyme is a heterodimer composed of Uba2 and Aos1 polypeptides, which resembles ubiquitin in its structure, its ability to be ligated to other proteins, as well as in the mechanism of ligation. However, SUMOlation does not mark proteins for degradation. It mediates ATP-dependent activation of UBL1 (ubiquitin-like 1) and formation of a thiolester with a conserved cysteine residue on SAE2. *Purity: 98% by SDS-PAGE.*	50 µg
NEW SUMO-Activating Enzyme E1 (Aos1/Uba2), Human	**662074**	SUMO (small ubiquitin-related modifier)-activating enzyme is a heterodimer composed of Uba2 and Aos1 polypeptides, which resembles ubiquitin in its structure, its ability to be ligated to other proteins, as well as in the mechanism of ligation. However, SUMOlation does not mark proteins for degradation. It mediates ATP-dependent activation of UBL1 (ubiquitin-like 1) and formation of a thiolester with a conserved cysteine residue on SAE2. *Purity: ≥98% by SDS-PAGE.*	25 µg
NEW Ubiquitin-Activating Enzyme E1, GST-Fusion, Human	**662071**	Required for initiating a multi-step pathway for the covalent linkage of ubiquitin to target proteins. It catalyzes the first step in the ubiquitin-protein isopeptide bond formation and is a critical component for the initiation of conjugation reactions *in vitro. Purity: ≥98% by SDS-PAGE.*	50 µg
NEW Ubiquitin-Activating Enzyme E1, Human	**662072**	Required for initiating a multi-step pathway for the covalent linkage of ubiquitin to target proteins. Catalyzes the first step in the ubiquitin-protein isopeptide bond formation and is a critical component for the initiation of conjugation reactions *in vitro. Purity: ≥98% by SDS-PAGE.*	25 µg
Ubiquitin-Activating Enzyme E1, Rabbit	**662070**	Catalyzes the first step in the ubiquitin-protein isopeptide bond formation. Activates ubiquitin monomer at its C-terminal cysteine residue by forming a high energy thioester bond. This activated ubiquitin is then transferred to lysine residues on target proteins via the E2/E3 conjugation cascade. Critical component for the initiation of conjugation reactions *in vitro*. *Purity: ≥95% by SDS-PAGE.*	10 µg

Ubiquitination Enzymes and Related Products *continued*

Product	Cat. No.	Comments	Size
Ubiquitin-Agarose	**662080**	Ubiquitin linked covalently to agarose beads via primary amine coupling thus leaving the C-terminus free. Useful for affinity binding of ubiquitin-activating enzyme (E1; Cat. No. 662070), ubiquitin carrier enzymes (E2s), ubiquitin ligases (E3s), ubiquitin C-terminal hydrolases (UCHs; Cat. No. 662090), and other enzymes and proteins that have an affinity for ubiquitin.	500 µl
Ubiquitin C-Terminal Hydrolase, Rabbit	**662090**	A member of the lower molecular weight ubiquitin C-terminal hydrolases (UCHs) involved in the hydrolysis of small C-terminal derivatives of ubiquitin that form non-specifically during protein ubiquitination. Also reported to play a significant role in the cleavage of the C-terminus of NEDD8, a ubiquitin-like protein that conjugates to nuclear proteins.	10 µg
Ubiquitin Conjugating Enzyme 2, GST-Fusion, Human, Recombinant, *E. coli*	**662111**	The human homolog to the yeast DNA repair gene *rad6*, E2-14K is induced by DNA damaging agents. E2-14K has been associated with muscle-induced wasting in cancer cachexia, diabetes, sepsis, and hyperthyroidism, promoting ubiquitin conjugation and degradation by the N-end rule pathway.	50 µg
Ubiquitin Conjugating Enzyme 5b, GST-Fusion, Human, Recombinant, *E. coli*	**662092**	E2s are a family of ubiquitin carrier proteins that transfer ubiquitin to the ε-lysine of proteins designated for degradation. UbcH5b is a human homolog of the yeast UBC4/5 family that has been shown to play many important regulatory roles in inflammation and cancer.	50 µg
Ubiquitin Conjugating Enzyme 6, His•Tag®, Human, Recombinant, *E. coli*	**662094**	E2s are a family of ubiquitin carrier proteins that transfer ubiquitin to the ε-lysine of proteins designated for degradation. A closely related member of the UbcH5 family, UbcH6 has been implicated in ER degradation of aberrant proteins. UbcH6 utilizes Nedd4 as its ubiquitin-protein ligase.	50 µg
Ubiquitin Conjugating Enzyme 10, His•Tag®, Human, Recombinant, *E. coli*	**662095**	E2s are a family of ubiquitin carrier proteins that transfer ubiquitin to the ε-lysine of proteins designated for degradation. UbcH10 in conjunction with the anaphase-promoting complex catalyzes the destruction of cyclin A and cyclin B, thus playing an important role in the control of the cells exit from mitosis.	50 µg
Ubiquitin Conjugating Enzyme 5a, Active Site Mutant, GST-Fusion, Human, Recombinant, *E. coli*	**662112**	The cysteine in the active site of UbcH5a has been mutated to serine to abolish the ability of the enzyme to transfer ubiquitin to an accepting E3/protein. Useful as an inactive control or to study protein-protein interactions.	50 µg
Ubiquitin Conjugating Enzyme Set	**662116**	Contains 10 µg each of the following ubiquitin conjugating (E2) enzymes: GST-Fusion UbcH2 (Cat. No. 662111), His•Tag® UbcH3, GST-Fusion UbcH5a (Cat. No. 662091), GST-Fusion UbcH5b (Cat. No. 662092), His•Tag® UbcH6 (Cat. No. 662094), UbcH7, and His•Tag® UbcH10 (Cat. No. 662095). Useful for selecting the appropriate enzyme for novel conjugations.	1 set
Ubiquitin Conjugating Enzyme Active Site Mutant Set	**662117**	Contains 10 µg each of the following ubiquitin conjugating (E2) enzymes mutated at the active site from cysteine to serine: GST-Fusion UbcH5a (Cat. No. 662112), His•Tag® UbcH6 (Cat. No. 662113), UbcH7 (Cat. No. 662114), and His•Tag® UbcH10 (Cat. No. 662115). Useful for selecting the appropriate negative control for novel conjugations.	1 set
Ubiquitin, GST-Fusion, Recombinant, *E. coli*	**662057**	Fully-functional ubiquitin with a glutathione S-transferase (GST) fusion at the N-terminus. Useful for ubiquitination of protein substrates and subsequent glutathione affinity purification of ubiquitinated molecules. Can also be used for immunodetection of conjugates using GST antibodies. Facilitates the visualization of polyUb chains due to larger ladder intervals.	1 mg
Ubiquitin, His•Tag®, Recombinant, *E. coli*	**662060**	Fully-functional ubiquitin with an N-terminal His•Tag® sequence. Useful for ubiquitination of protein substrates and subsequent metal chelate affinity purification of ubiquitinated molecules. Can also be used for immunodetection of conjugates using 6-His-specific antibodies.	1 mg

Ubiquitination Enzymes and Related Products *continued*

Product	Cat. No.	Comments	Size
Ubiquitin K48R, His•Tag® Fusion, Human, Recombinant, *E. coli*	**662064**	Mutant ubiquitin featuring a Lys^{48} to Arg^{48} mutation that prevents the formation of polyUb chains via Lys^{48} linkages with other ubiquitin molecules. Forms an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins (mono-ubiquitination). Useful for the reduction of polyUb chain length and conjugation rates, and for determining lysine position linkage. A potent suppressor of TNF-α release by macrophages.	1 mg
Ubiquitin, Methylated, Bovine	**662063**	Reductively methylated ubiquitin that cannot form polyUb linkages with other ubiquitin molecules. Forms an E1-catalyzed active thioester at the C-terminus allowing the molecule to be transferred to the lysines of substrate proteins (mono-ubiquitination). Useful for the reduction in polyUb chain length as well as conjugation rates.	1 mg

Proteasome Activator/Substrates

Product	Cat. No.	Comments	Size
PA28 Activator, Rabbit	**506280**	Mixture of two homologous subunits (α and β) that stimulates the 20S proteasome. PA28 also contains a protein known as PA28γ of undefined function. Enhances hydrolysis of peptide substrates by the proteasome (peptidase activity) but not its ability to degrade ubiquitinated proteins. Also modulates the antigen processing function of proteasome.	10 µg
Proteasome Substrate I, Fluorogenic (Z-LLL-AMC)	**539140**	Fluorogenic proteasome substrate. *Purity:≥95% by HPLC. Excitation max:~380 nm, Emission max:~460 nm.*	5 mg
Proteasome Substrate II, Fluorogenic (Z-LLE-AMC)	**539141**	Fluorogenic proteasome substrate. *Purity:≥98% by HPLC. Excitation max:~380 nm, Emission max:~460 nm.*	5 mg
Proteasome Substrate III, Fluorogenic (SUC-LLVY-AMC)	**539142**	Fluorogenic proteasome substrate. Also acts as a substrate for calpain, chymotrypsin, and ingensin. *Purity:≥98% by HPLC. Excitation max:~380 nm, Emission max:~460 nm.*	5 mg
Proteasome Substrate IV, Fluorogenic (Z-VKM-AMC)	**539143**	Fluorogenic substrate for Alzheimer's disease amyloid A4-generating enzymes and for the proteasome. *Purity:≥98% by HPLC. Excitation max:~380 nm, Emission max:~460 nm.*	5 mg
Proteasome Substrate V, Fluorogenic (Z-GGL-AMC)	**539144**	A fluorogenic substrate for the detection of 20S proteasome. *Purity:≥98% by HPLC. Excitation max:~380 nm, Emission max:~460 nm.*	5 mg
Proteasome Substrate VI, Fluorogenic (Z-ARR-AMC, 2HCl)	**539149**	A fluorogenic substrate probe useful for assaying trypsin-like activity of proteasome. Also reported to be a specific substrate for cathepsin B. *Purity:≥99% by TLC. Excitation max:360-380 nm, Emission max:430-460 nm.*	5 mg
Proteasome Substrate VII, Fluorogenic (ABz-GPALA-NBA)	**539151**	A highly sensitive fluorescence resonance energy transfer (FRET) peptide substrate that is useful for continuously monitoring the branched chain amino acid preferring peptidase (BrAAP) activity of the 20S proteasome (K_{cat}/K_m = 13,000 $M^{-1}s^{-1}$ at 37˚C, pH 7.8). The 20S proteasome cleaves the substrate at the Leu-Ala bond resulting in fluorescence increase. *Purity: ≥95% by HPLC. Excitation max:~340 nm, Emission max:~415 nm.*	1 mg
Ubiquitin-AMC (Ub-AMC)	**662075**	Fluorogenic substrate for ubiquitin hydrolases. Ub-AMC is an excellent substrate for UCH-L3 (Cat. No. 662090; K_m = 39 nM) and for the Isopeptidase T (Cat. No. 419700; K_m = 0.17 - 1.4 mM). Useful for studying ubiquitin hydrolases when detection sensitivity or continuous monitoring of activity is required. *Purity:≥95% by HPLC. Excitation max:~340 nm, Emission max: ~425 nm.*	25 µg

Proteasome and Ubiquitination Inhibitors

Product	Cat. No.	Cell-permeable?	Reversible?	Comments	Size
Aclacinomycin A, *Streptomyces galilaeus*	**112270**	No	Yes	A non-competitive inhibitor of the chymotrypsin-like activity of the 20S proteasome (IC_{50} = ~52 μM).	50 mg
AdaAhX$_3$L$_3$VS	**114802**	Yes	No	Inhibits chymotrypsin-like (IC_{50} = 0.05 – 0.01 μM), trypsin-like (IC_{50} = 1.0 – 5.0 μM), and PGPH (IC_{50} = 0.5 – 1.0 μM) activities of the 20S proteasome.	250 μg
AdaLys(bio)AhX$_3$L$_3$VS	**114803**	Yes	No	Inhibits chymotrypsin-like (IC_{50} = 0.05 – 0.1 μM), trypsin-like (IC_{50} = 5.0 – 10.0 μM), and PGPH (IC_{50} = 2.0 – 5.0 μM) activities of the 20S proteasome in living cells. Useful for the detection of catalytic β-subunits of both constitutive proteasome and immunoproteasome through immunoblotting.	250 μg
ALLN (Calpain Inhibitor I)	**208719**	Yes	Yes	Inhibits chymotrypsin-like activity of the proteasome (K_i = 5.7 μM). Also inhibits the activation of calpain I, calpain II, cathepsin B, and cathepsin L.	5 mg 25 mg
Epoxomicin, Synthetic	**324800**	Yes	No	Inhibits chymotrypsin-like, trypsin-like and peptidylglutamyl peptide hydrolyzing (PGPH) activities of the proteasome.	100 μg
NEW InSolution™ Epoxomicin, Synthetic	**324801**	Yes	No	Supplied as a 0.812 mM (50 μg/111 μl) solution of Epoxomicin, Synthetic (Cat. No. 324800) in DMSO.	50 μg
NEW Hdm2 E3 Ligase Inhibitor	**373225**	Yes	Yes	A cell-permeable, reversible inhibitor of hdm2 E3 ligase that is shown to block hdm2-mediated ubiquitination of p53 (IC_{50} = 12.7 μM using Ub-Ubc4 as the donor substrate). The inhibition is non-competitive with respect to either the donor or acceptor substrate.	5 mg
Lactacystin, Synthetic	**426100**	Yes	No	A potent and selective proteasome inhibitor. Inhibits the chymotrypsin and trypsin-like peptidase activities of proteasomes. Also inhibits cathepsin A.	200 μg
clasto-Lactacystin-β-Lactone	**426102**	Yes	No	A potent and selective proteasome inhibitor. Inhibits the chymotrypsin and trypsin-like peptidase activities of proteasomes. Also inhibits cathepsin A.	100 μg
α-Methylomuralide	**426104**	Yes	No	An α-methyl analog of *clasto*-Lactacystin β Lactone (Omuralide, Cat. No. 426102) that displays improved hydrolytic stability. Reported to be a potent, selective, and irreversible inhibitor of proteasome function (k_{inact} chymotrypsin-like peptidase activity of purified 20S proteasome from bovine brain = 2300 $M^{-1}s^{-1}$ for α-Methylomuralide *vs.* 3060 $M^{-1}s^{-1}$ for Omuralide).	100 μg
MG-115 (Z-LL-Nva-CHO)	**474780**	Yes	Yes	Potent proteasome inhibitor (IC_{50} = 21 nM and 35 nM for 20S and 26S proteasomes, respectively). Inhibits chymotrypsin-like activity of the proteasomes.	5 mg
MG-132 (Z-LLL-CHO)	**474790**	Yes	Yes	Inhibits chymotrypsin-like activity of the proteasomes (K_i = 4 nM).	1 mg 5 mg
NEW InSolution™ MG-132	**474791**	Yes	Yes	A 10 mM solution of MG-132 (Cat. No. 474790) in anhydrous DMSO.	1 mg
NLVS	**482240**	Yes	No	Inhibits chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptidase activities of proteasomes.	500 μg
NP-LLL-VS	**492025**	Yes	No	An intermediate that can be used to prepare radiolabeled ^{125}I-NIP-L$_3$VS for proteasome inhibition studies. NIP-L$_3$VS acts by covalently modifying the active site threonine of the catalytic β-subunit of the proteasome.	500 μg
PR-11	**529643**	Yes	Yes	The active sequence derived from the first 11-amino acids of PR-39 (Cat. No. 529645) that acts as a selective inhibitor of proteasome-mediated IκBα degradation (25% inhibition reported at 500 nM).	1 mg
PR-39, Porcine, Synthetic	**529645**	Yes	Yes	A member of the proline/arginine-rich group of cathelicidin peptides that reversibly binds to the α-7 subunit of 20S proteasome and blocks degradation of NF-κB by the ubiquitin-proteasome pathway without affecting overall proteasome activity.	100 μg

Proteasome and Ubiquitination Inhibitors, *continued*

Product	Cat. No.	Cell-permeable?	Reversible?	Comments	Size
Proteasome Inhibitor I (PSI)	**539160**	Yes	Yes	Inhibits chymotrypsin-like activity of the proteasomes.	1 mg 5 mg
NEW InSolution™ Proteasome Inhibitor I	**539161**	Yes	Yes	Supplied as a 50 mM (5 mg/162 µl) solution of Proteasome Inhibitor I (Cat. No. 539160) in DMSO.	5 mg
Proteasome Inhibitor II (Z-LLF-CHO)	**539162**	Yes	Yes	Inhibits chymotrypsin-like activity of the proteasomes (K_i = 460 nM).	1 mg 5 mg
Proteasome Inhibitor III [Z-LLL-B(OH)$_2$]	**539163**	Yes	Yes	Inhibits chymotrypsin-like activity of the proteasomes (K_i = 30 pM).	100 µg
Proteasome Inhibitor IV (Z-GPFL-CHO)	**539175**	Yes	Yes	K_is = 1.5 µM for branched chain amino acid preferring, 2.3 µM for small neutral amino acid preferring, and 40.5 µM for chymotrypsin-like activities; IC_{50} = 3.1 µM for PGPH activity. Only weakly inhibits trypsin-like proteasomal activity.	5 mg
Ro106-9920	**557550**	Yes	No	A highly selective, irreversible inhibitor of IκBαee ubiquitination (IC_{50} = 2.3 µM). Blocks NF-κB-dependent cytokine expression in human PBMNs (IC_{50} ~700 nM for TNF-α, IL-1β, and IL-6 inhibition) and rats.	1 mg 5 mg
Ro106-9920, Control	**557551**	Yes	—	A negative control compound for Ro106-9920 (Cat. No. 557550) (IκBαee ubiquitination IC_{50} > 80 µM).	1 mg
Tyropeptin A, Synthetic	**657008**	Yes	Yes	IC_{50} = 100 ng/ml for chymotrypsin-like, and 1.5 µg/ml for trypsin-like activities. No effect on PGPH activity even at a concentration of 100 µg/ml.	1 mg
Ubiquitin Aldehyde	**662056**	No	Yes	Potent and specific inhibitor of multiple ubiquitin hydrolases involved in pathways of intracellular protein modification and turnover.	50 µg
NEW UCH-L1 Inhibitor	**662086**	Yes	Yes	A potent, reversible, competitive, and active site-directed inhibitor of UCH-L1 (K_i = 400 nM; IC_{50} = 880 nM) with ~28-fold greater selectivity over UCH-L3 (Cat. No. 662090).	10 mg
NEW UCH-L3 Inhibitor	**662089**	Yes	No	A selective and potent inhibitor of UCH-L3 (IC_{50} = 600 nM) with ~125-fold greater selectivity over UCH-L1 (IC_{50} = 75 µM).	10 mg
YU 101	**688500**	Yes	No	Inhibits chymotrypsin-like activity of the proteasomes. Only weakly inhibits the trypsin-like and peptidylglutamyl peptide hydrolyzing (PGPH) activities of the proteasome.	100 µg

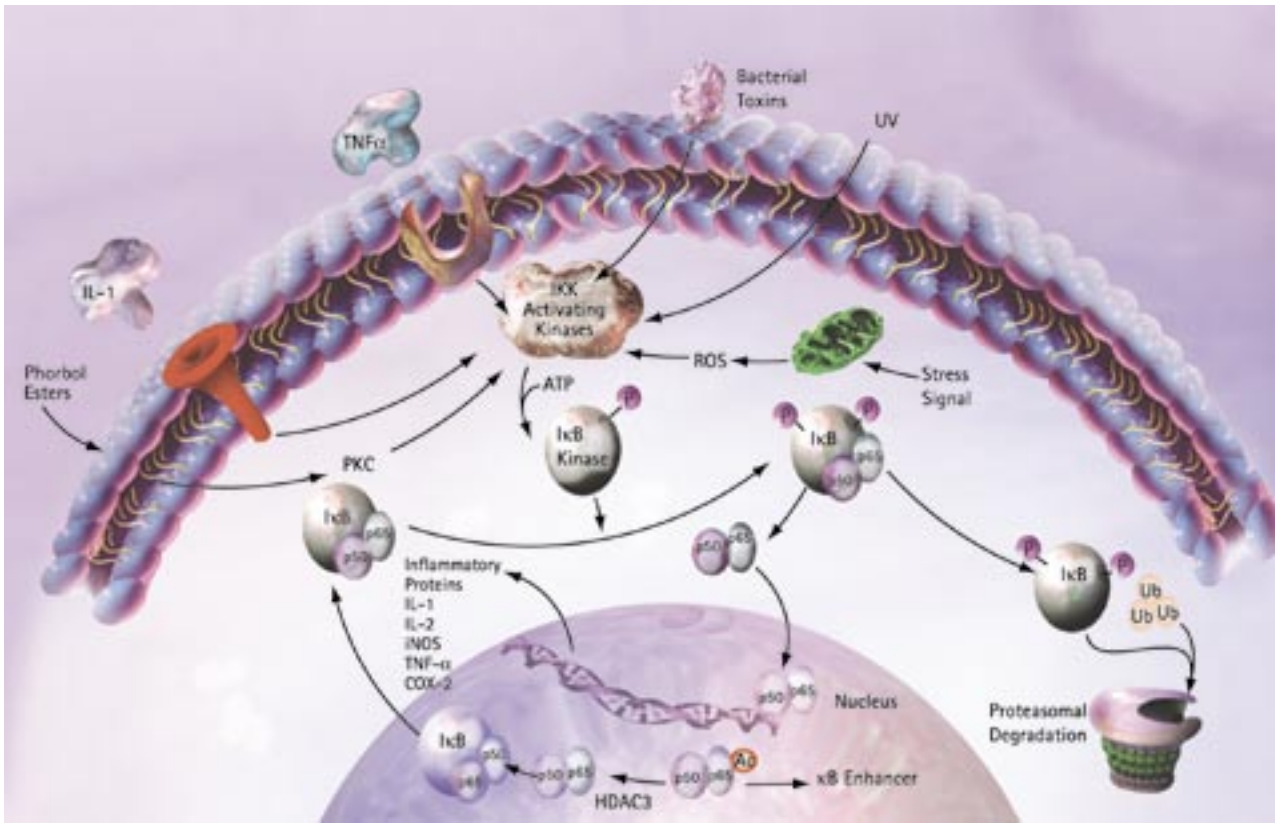
NF-κB: A Therapeutic Target for Inflammation and Cancer

Chandra Mohan, Ph.D.
Merck Biosciences

The eukaryotic nuclear factor κB (NF-κB) plays an important role in inflammation, autoimmune response, cell proliferation, and apoptosis by regulating the expression of genes involved in these processes. Five members of the NF-κB family have been identified: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and c-Rel. They share a highly conserved Rel homology domain (RHD), which is responsible for DNA binding, dimerization, and interaction with IκB. The C-terminal region of RHD contains a nuclear localization sequence (NLS) that remains inactive in non-stimulated cells through binding to IκB proteins. The p50/RelA(p65) heterodimer is the major Rel/NF-κB complex in most cells. RelB can act either as a transcriptional activator or as a repressor of NF-κB-dependent gene expression. It acts as an activator when it associates with p50 or p52. However, its inhibitory effect has been attributed to the formation of the RelA(p65):RelB heterodimer that does not bind to κB sites. Studies using NIH 3T3 cells have also shown that RelA(p65):RelB heterodimers are not regulated by IκB and are located in both the cytoplasm and the nucleus.

The activity of NF-κB is tightly regulated by its interaction with inhibitory IκB proteins. In most resting cells, NF-κB is sequestered in the cytoplasm in an inactive form associated with inhibitory molecules such as IκBα, IκBβ, IκBε, p105, and p100. This interaction blocks the ability of NF-κB to bind to DNA and results in the NF-κB complex being primarily localized to the cytoplasm due to a strong nuclear export signal in IκBα. Following exposure to inflammatory cytokines, UV light, reactive oxygen species, or bacterial and viral toxins, the NF-κB signaling cascade is activated, leading to the complete degradation of IκB. This allows the translocation of unmasked NF-κB to the nucleus where it binds to the enhancer or promoter regions of target genes and regulates their transcription. In the nucleus, acetylation of NF-κB determines its active or inactive state. p300 and CBP acetyltransferases play a major role in the acetylation of RelA(p65), principally targeting $Lys^{218, 221, 310}$ for modification. Acetylated NF-κB is active and is resistant to the inhibitory effects of IκB. However, when histone deacetylase 3 (HDAC3) deacetylates NF-κB, IκB readily binds to NF-κB and causes its translocation into the cytoplasm. Here HDAC3 serves as an intranuclear molecular switch that turns off the biological processes triggered by NF-κB. One of the target genes activated by NF-κB is that encoding IκBα. Newly synthesized IκBα can enter the nucleus, remove NF-κB from DNA, and export the complex back to the cytoplasm to restore its original latent state.

The activation of NF-κB by extracellular inducers depends on the phosphorylation and subsequent degradation of IκB proteins. Activation of NF-κB is achieved through the action of a family of serine/threonine kinases known as IκB kinase (IKK). The IKK contains two catalytic subunits (IKKα and IKKβ) and a regulatory/adapter protein NEMO (also known as IKKγ). IKK activity and NF-κB activation are largely dependent on the integrity of NEMO and IKKβ. Cells devoid of IKKα can still show normal induction of NF-κB-DNA-binding in response to stimuli. IKKα and IKKβ phosphorylate IκB proteins and the members of the NF-κB family. All IκB proteins contain two conserved serine residues within their N-terminal area, which are phosphorylated by IKK. IKKα and IKKβ share about 50% sequence homology and can interchangeably phosphorylate $Ser^{32/36}$ of IκBα, and $Ser^{19/23}$ of IκBβ. These phosphorylation events lead to the immediate polyubiquitination of IκB proteins and rapid degradation by the 26S proteasome complex.



NF-κB Signaling Pathways

The Rel/NF-κB signal transduction pathway is misregulated in a variety of human cancers, especially those of lymphoid cell origin. Several human lymphoid cancer cells are reported to have mutations or amplifications of genes encoding NF-κB transcription factors. In most cancer cells NF-κB is constitutively active and resides in the nucleus. In some cases, this may be due to chronic stimulation of the IKK pathway, while in others the gene encoding IκBα may be defective. Such continuous nuclear NF-κB activity not only protects cancer cells from apoptotic cell death, but also may enhance their growth activity. Hence, constitutive NF-κB expression is considered as a reliable factor to predict the metastatic potential of tumors, indicating early therapy for NF-κB inhibition. Designing anti-tumor agents to block NF-κB activity or to increase their sensitivity to conventional chemotherapy may have great therapeutic value. Researchers have explored various possibilities of interfering with NF-κB activation and its binding to DNA. They include inhibition of NF-κB dimerization, inhibition of NF-κB activation, and the use of proteasome inhibitors that block IκB degradation and NF-κB nuclear transformation. Since IKKα and IKKβ are not shown to phosphorylate any protein not involved in NF-κB signaling, they are considered as important targets for the development of chemotherapeutic agents.

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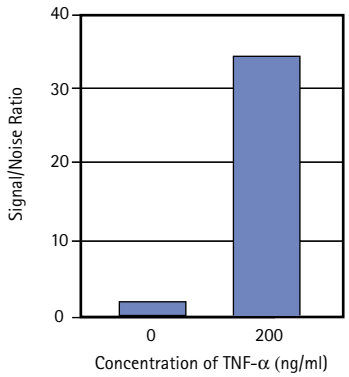
NoShift™ Transcription Factor Assay Kit

Non-radioactive, rapid, versatile, colorimetric detection system

Measure the activation of DNA-binding proteins in less than five hours using the versatile 96-well NoShift™ Transcription Factor Assay Kit and NoShift NF-κB (p65) Reagents. The assay kit, an EMSA alternative, consists of the assay buffer, a streptavidin coated plate, and TMB substrate. The reagent kit include the NF-κB consensus binding sequence (as a biotinylated oligonucleotide), NF-κB antibody, and HRP detection antibody, as well as positive and negative controls.



NoShift™ NF-κB. After a 30-minute stimulation with 200 ng/ml TNF-α, nuclear extracts from HeLa cells were prepared with NucBuster™ reagent. The nuclear extract was analyzed using NoShift NF-κB (p65) Reagents.

NoShift™ Transcription Factor Assay Kit

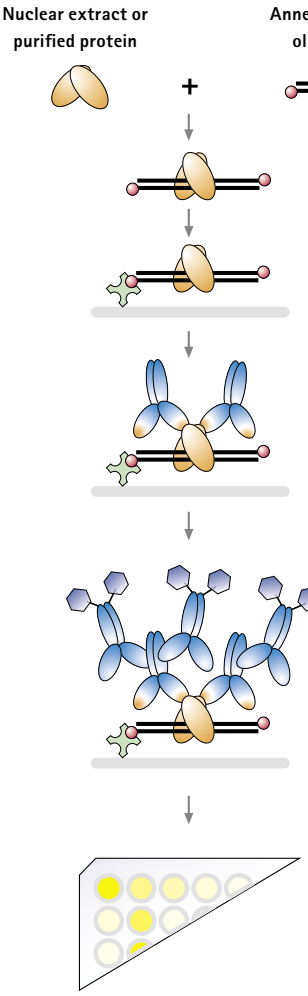
Cat. No. 71377-3 1 kit

NoShift™ NF-κB (p65) Reagents

Cat. No. 71518-3 1 kit

NoShift™ Transcription Factor Assay Kit plus NucBuster

Cat. No. 71378 1 kit



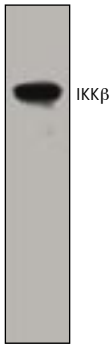
1. Anneal two oligonucleotides that define a binding site (at least one oligonucleotide should be end labeled with biotin).
2. Incubate dsDNA with the extract or protein of interest for 30 min on ice.
3. Transfer the samples to a streptavidin plate. Incubate for 1 h at 37˚ C.
4. Wash.
5. Add antibody specific for the DNA-binding protein of interest to the samples, incubate for 1 h at 37˚C.
6. Wash.
7. Add a secondary antibody conjugated with horseradish peroxidase (HRP). Incubate the samples for 30 min at 37˚C.
8. Wash.
9. Add TMB Substrate and incubate for 10-30 min to develop colorimetric signal before quenching the reaction with 1 N HCl.
10. Read absorbance at 450 nm and record results.

The NoShift™ assay is a microassay plate–based test to identify proteins that bind to a specific DNA sequence. One advantage of this plate-based assay over traditional gel shift assays is the remarkable specificity of the test. The shift in mobility of a DNA probe in an EMSA indicates that some protein in a crude extract binds, but the identity of the protein is unknown unless a supershift is performed with a protein-specific antibody. The NoShift assay has dual specificity: that of the protein for the DNA probe and of the antibody for the interacting protein. The convenient 96-well format of the NoShift assay permits screening for multiple DNA binding proteins in the same plate. The basic NoShift kit consists of a streptavidin-coated microassay plate with sealers; buffers for binding, washing, and dilution; and TMB Substrate. Also available is the NoShift Transcription Factor Assay Kit Plus NucBuster™, which includes the microassay plate and sealers, buffers, and substrate as well as a complete NucBuster Protein Extraction Kit to prepare nuclear extracts in less than 30 minutes. In addition to NF-κB reagent, four transcription factor specific reagent kits are also offered for use with the NoShift assay kits. The c-Fos, Sp1, ER-α, and HIF-α reagent kits each contain biotinylated oligonucleotides that include a consensus recognition sequence, specific and non-specific competitors, a transcription factor–specific antibody, secondary antibody conjugated to HRP, and positive control nuclear extract. The NoShift™ assay kits and convenient reagent kits offer a fast, sensitive, nonradioactive alternative to gel shift assays.

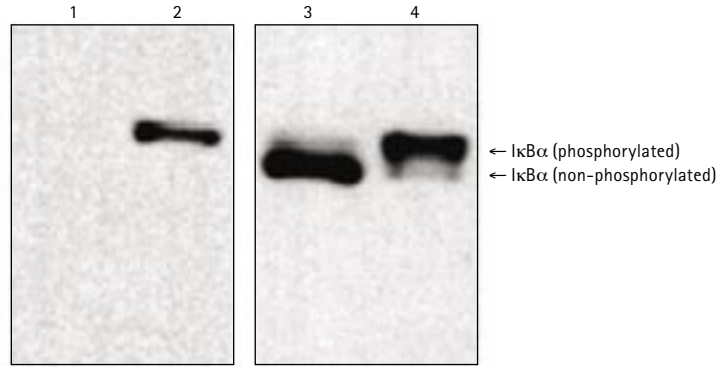
Antibodies for NF-κB Pathway Research

Product	Cat. No.	Comments	Applications	Size
Anti-cRel Rabbit pAb	**PC139**	Polyclonal IgG, diluted serum. Immunogen used was a synthetic peptide corresponding to a region near the C-terminal domain of the human cRel. Detects human NFκB cRel.	ELISA, GS, IB, IP	20 µl 100 µl
Anti-IκBα, Cleaved, Mouse mAb	**OP197**	Monoclonal IgG_1, purified. Detects the ~36 kDa amino-terminal truncated IκBα (DN-IκBα)that is resistant to degradation.	IB	100 µg
Anti-IκBα (Ab-1), Rabbit pAb	**PC381**	Polyclonal IgG, purified. IκBα migrates as a doublet or triplet as a result of variable phosphorylation.	IB	100 µg
Anti-IKKβ Mouse mAb	**OP134**	Monoclonal IgG_1, purified. Immunogen used was a His•Tag® full-length human IKKβ protein. Recognizes an ~87 kDa band in Daudi cell lysates.	IB	100 µg
Anti-IKKγ (400-416) Rabbit pAb	**400003**	Polyclonal IgG, affinity purified. Recognizes the ~52 kDa protein in cell lysates. Does not cross-react with IKKα or IKKβ. IKKγ phosphorylates IκB thus mediates NF-κB activity.	IB	100 µg
Anti-IKKε/IKK-*i* (701-716) Rabbit pAb	**400004**	Polyclonal IgG, affinity purified. Recognizes an ~80 kDa protein in cell human lysates.	IB	100 µg
Anti-Interleukin-1 Receptor-Associated Kinase-M (581-596) Rabbit pAb	**407160**	Polyclonal IgG, affinity purified. Recognizes the ~68 kDa protein. Does not cross-react with IRAK or IRAK2.	IB	100 µg
Anti-NAK/TBK1 (712-727) Rabbit pAb	**477740**	Polyclonal IgG, affinity purified. Recognizes the ~84 kDa NAK protein. Does not cross-react with IKKα, IKKβ, IKKγ, or IKKε.	IB	100 µg
Anti-NF-κB (p50) (Ab-1) Rabbit pAb	**PC136**	Polyclonal IgG, undiluted serum. Detects the human NF-κB1 p50 and p150 proteins. Reacts with p50:p50 homodimers and p50:p65 heterodimers in gel shift assays.	ELISA, GS	20 µl 100 µl
Anti-NF-κB (p65, RelA) (Ab-1), Rabbit pAb	**PC137**	Polyclonal IgG, undiluted serum. Detects p65 (Rel A) in human and mouse.	GS, IB, IP	20 µg 100 µl
NF-κB Antibody Sampler Kit	**ASK20**	20 µl samples of various NF-κB antisera. Includes Anti-NF-κB (Cat. Nos. PC136, PC137, and PC139) and Anti-IκBα (Cat. No. PC142). Please see individual product descriptions for more information.		1 each
PhosphoDetect™ Anti-IκBα, ($pSer^{32/36}$) Mouse mAb	**OP142**	Monoclonal IgG_1, purified. Detects a ~42 kDa band corresponding to the phosphorylated form of IκBα.	IB, IP	100 µg
PhosphoDetect™ Anti-IκBα, ($pSer^{32}$) Rabbit pAb	**400002**	Polyclonal IgG, purified. Reacts with phosphorylated IκBα. Does not react with IκBβ and IκBε.	IB, IC, IP	100 µl

ELISA = enzyme-linked immunosorbent assay • FS = frozen section • GS = gel shift assays • IB = immunoblotting
IC = immunocytochemistry • IH = immunohistochemistry • IP = immunoprecipitation • PS = paraffin sections



Detection of human IKKβ by immunoblotting.
Samples: Whole cell lysates (30 µg) from Daudi cells. Primary antibody: Anti-IKKβ Mouse mAb (10AG2) (Cat. No. OP134) (1:1000).



Detection of human IκBα, phosphorylated on $Ser^{32/36}$, by immunoblotting.
Sample: Whole cell lysate from Jurkat cells treated with ALLN (Cat. No. 208719) (100 µg/ml for 30 min.), followed by incubation with TNF-α (1 nM, lanes 2, 4) or without TNF-α (lanes 1, 3). Primary antibody: PhosphoDetect Anti- IκBα, Mouse mAb (Cat. No. OP142) (1:500 – 1:2000). Detection: chemiluminescence .

NF-κB Activation Inhibitors and Related Products

Product	Cat. No.	Comments	Size
NEW Acetyl-11-keto-β-Boswellic Acid, *Boswellia serrata*	**110123**	A cell-permeable pentacyclic triterpene of the ursane type that displays antitumor and anti-inflammatory properties. Shown to block the phosphorylation and degradation of IκBα and inhibit NF-κB-mediated gene transcription in chemoresistant androgen-independent PC-3 prostate cells (~10 μM) and in xenografted mice.	5 mg
NEW Andrographolide	**172060**	A bicyclic diterpenoid lactone that displays anti-viral, anti-inflammatory, anti-apoptotic, and anti-hyperglycemic properties. Acts as an irreversible antagonist of NF-κB and AP-1 ($IC_{50} \leq 15$ μM) activation, and prevents *in vitro* and *in vivo* T cell activation. Exerts no effect on IκBα degradation, p50 and p65 nuclear translocation. Inhibits iNOS and Mac-1 expression and ROS production, and prevents endotoxin shock in mouse model. Further, reported to activate PI3K/Akt pathway.	50 mg
BAY 11-7082	**196870**	Potential anti-inflammatory agent. Selectively and irreversibly inhibits the TNFα-inducible phosphorylation of IκBα (IC_{50} = 10 μM) without affecting the constitutive IκBα phosphorylation. Decreases nuclear translocation of NF-κB and inhibits TNF-α-induced surface expression of the endothelial-leukocyte cell adhesion molecules E-selectin, VCAM-1, and ICAM-1.	10 mg
NEW InSolution™ BAY 11-7082	**196871**	Supplied as a 100 mM (10 mg/483 μl) solution of BAY 11-7082 (Cat. No. 196870) in DMSO. *Purity ≥ 95% by HPLC.*	10 mg
BAY 11-7085	**196872**	Exhibits biological properties similar to that of BAY 11-7082 (Cat. No. 196870). BAY 11-7085 has also been shown to have potent anti-inflammatory properties *in vivo*.	10 mg
CAPE	**211200**	An active component of propolis from honeybee hives with antiviral, anti-inflammatory, and immunomodulatory properties. Has been shown to act as a potent and specific inhibitor of NF-κB activation.	25 mg
(E)-Capsaicin	**211274**	An active constituent of cayenne pepper that has anti-nociceptive and anti-inflammatory effects. Inhibits NF-κB activation by TNF.	100 mg
Gliotoxin, *Gladiocladium fimbriatum*	**371715**	An immunosuppressive secondary metabolite produced by several pathogenic fungi that specifically inhibits NF-κB activation in B and T cells at nanomolar concentrations.	1 mg
Helenalin, A. *chamissonis* ssp. *foliosa*	**374000**	A cell-permeable pseudoguainolide sesquiterpenoid lactone that inhibits NF-κB-DNA binding activity by selectively alkylating the p65 subunit of NF-κB. Does not inhibit IκB degradation or NF-κB nuclear translocation.	500 μg
Hypoestoxide, *Hypoestes rosea*	**401006**	A naturally occurring, cell-permeable diterpene with anti-inflammatory properties. Acts as a selective and direct inhibitor of IκB kinase (IC_{50} = 24 μM) in TNF-α stimulated HeLa cells thereby prevents NF-κB activation.	1 mg
IκB Kinase Inhibitor Peptide, Cell-Permeable	**401477**	A 14-amino acid peptide corresponding to the active IκB phosphorylation recognition sequence, linked to the hydrophobic region of the fibroblast growth factor signal peptide to aid in cellular delivery. Specifically inhibits LPS-induced IκB degradation by IκB kinases (IKK) in RAW 264.7 cells (< 50 μg/ml) and prevents NF-κB activation.	1 mg
IκB Kinase Inactive Control Peptide, Cell-Permeable	**401478**	An inactive control for IκB Kinase Inhibitor Peptide (Cat. No. 401477). Corresponds to the mutated recognition sequence of IκB (Ser^{32} → Ala and Ser^{36} → Ala), linked to the hydrophobic region of the fibroblast growth factor signal peptide to aid in cellular delivery. Does not have any inhibitory effect on LPS-induced IκB degradation by IκB kinases (IKK) in RAW 264.7 cells at 50 μg/ml.	1 mg
NEW IKK Inhibitor II, Wedelolactone	**401474**	The naturally isolated active ingredient of the herbal medicine, *Eclipta alba*, that acts as a selective and irreversible inhibitor of IKKα and β kinase activity (IC_{50} < 10 μM). Inhibits NF-κB-mediated gene transcription in cells by blocking the phosphorylation and degradation of IκB.	1 mg
NEW IKK Inhibitor III, BMS-345541	**401480**	A cell-permeable, potent, selective, and allosteric site-binding inhibitor of IKK-2 (IC_{50}~300 nM). Exhibits ~10 fold greater selectivity over IKK-1 (IC_{50} ~4 μM).	1 mg

NF-κB Activation Inhibitors and Related Products *continued*

Product	Cat. No.	Comments	Size
NEW IKK-2 Inhibitor, SC-514	**401479**	A cell-permeable, potent, reversible, ATP-competitive, and highly selective inhibitor of IKK-2 (IC_{50} ~3-12 μM for IKK-2 homodimer, IKK-1/IKK-2 heterodimer, and IKK-2). Shown to specifically block NF-κB-dependent gene expression, but not MAP kinase pathways, in stimulated RASF synovial fibroblast cells. Does not inhibit the phosphorylation and activation of the IKK complex.	1 mg
NEW IKK-2 Inhibitor IV	**401481**	A cell-permeable, potent, and selective inhibitor of IKK-2 (IC_{50} = 18 nM) with selectivity over IKK-1, JNK, and p38 MAPK.	500 µg
NEW IKK-2 Inhibitor V	**401482**	A cell-permeable salicylamide compound that acts as an IKK-2 inhibitor by selectively blocking IκBα phosphorylation (IC_{50} ~250 nM) and prevents the induction of NF-κB p65 nuclear translocation. Shown to offer cardioprotection by reducing IL-1β and MCP-1 production (IC_{50} < 1 μM) in cardiomyocytes, and ameliorate insulin resistance in KKA^{y} mice by regulating adiponectin release.	5 mg
NEW IKK-2 Inhibitor VI	**401483**	An ureido-thiophenecarboxamide compound that acts as a potent inhibitor of IKK-2 with IC_{50} of 13 nM.	1 mg
Isohelenin, *Inula* sp.	**416157**	A cell-permeable sesquiterpene lactone with anti-inflammatory properties. Acts as a highly specific, potent, and irreversible inhibitor of NF-κB activation by preventing IκBα degradation. Does not affect the DNA binding activity of activated NF-κB or inhibit Fyn and Src kinase activities.	1 mg
Kamebakaurin, *Isodon japonicus*	**420340**	A potent, irreversible inhibitor of NF-κB activation (100% inhibition at ~26.6 μM) that acts by directly targeting the DNA-binding activity of p50 and blocking the expression of anti-apoptotic NF-κB target genes. Does not affect the induced degradation of IκBα and nuclear translocation of NF-κB.	500 µg
NEMO-Binding Domain Binding Peptide, Cell-Permeable	**480025**	A cell-permeable antennapedia NBD (NEMO binding domain) (wild type) fusion peptide that exhibits anti-inflammatory activity in mouse model of acute inflammation. NBD is an amino-terminal α-helical region of the NEMO (NF-κB essential modifier; IKKγ) associated with a carboxyl-terminal segment of IKKα and IKKβ. Blocks the association of NEMO with the IKK complex and prevents NF-κB activation.	500 µg
NEMO-Binding Domain Binding Peptide, Cell-Permeable, Negative Control	**480030**	A cell-permeable, antennapedia-NBD mutated (Trp^{739} → Ala and Trp^{741}→ Ala) fusion peptide analog of NEMO-Binding Domain Binding Peptide (Cat. No. 480025) that serves as a negative control. Reported to be defective in binding to NEMO.	500 µg
NEW NF-κB Activation Inhibitor	**481406**	A cell-permeable quinazoline compound that acts as a potent inhibitor of NF-κB transcriptional activation (IC_{50} = 11 nM in Jurkat cells) and LPS-induced TNF-α production (IC_{50} = 7 nM in murine splenocytes). Does not exhibit cellular toxicity at concentrations required for inhibition of NF-κB transcriptional activation (IC_{50} > 10 μM) or TNF-α production (IC_{50} >10 μM).	1 mg
NF-κB SN50, Cell-Permeable Inhibitor Peptide	**481480**	Contains the nuclear localization sequence (NLS) of the transcription factor NF-κB p50 linked to the hydrophobic region (h-region) of the signal peptide of Kaposi fibroblast growth factor (K-FGF). The peptide N-terminal K-FGF h-region confers cell-permeability, while the NLS (360-369) inhibits translocation of the NF-κB active complex into the nucleus.	500 µg
NF-κB SN50M, Cell-Permeable Inactive Control Peptide	**481486**	An inactive control for SN50 peptide (Cat. No. 481480). Corresponds to the SN50 peptide sequence with substitutions of Lys^{363} for Asn and Arg^{364} for Gly in the NLS region.	500 µg
Parthenolide, *Tanacetum parthenium*	**512732**	A sesquiterpene lactone with anti-inflammatory, antisecretory, and spasmolytic properties. Inhibits NF-κB and activation of MAP kinase.	50 mg
PPM-18	**529570**	A novel, cell-permeable, anti-inflammatory agent that inhibits the expression of inducible nitric oxide synthase (iNOS; IC_{50} ~5 μM). Acts by blocking the activation of NF-κB *in vitro* and *in vivo*.	10 mg
Sulfasalazine	**573500**	An anti-inflammatory agent that acts an inhibitor of glutathione S-transferase (IC_{50} = 10 μM in H-69 cell line). Prevents NF-κB activation and induces apoptosis in T lymphocytes.	100 mg

NF-κB Activation Inhibitors and Related Products *continued*

Product	Cat. No.	Comments	Size
TIRAP Inhibitor Peptide, Cell-Permeable	**613570**	A synthetic, cell-permeable peptide corresponding to mouse toll-interleukin 1 receptor (TIR) domain-containing adapter protein 138-151 (TIRAP) fused to the *Drosophila* Antennapedia sequence. Specifically inhibits LPS-induced, but not CpG-induced, NF-κB activation, PKR phosphorylation, and JNK phosphorylation in RAW.κB cells at ~ 40 μM. Also reported to block IκBα degradation.	1 mg
TIRAP Inhibitor Peptide, Control, Cell-Permeable	**613571**	A cell-permeable synthetic peptide containing mouse toll-interleukin 1 receptor (TIR) domain-containing adapter protein 151-138 reverse sequence (TIRAP) fused to the *Drosophila* Antennapedia sequence. Serves as a control for TIRAP Inhibitor Peptide (Cat. No. 613570).	1 mg

Fusion Tag Antibodies

GST•Tag™ Monoclonal Antibody

Sensitive, specific detection of GST•Tag fusion proteins

Specificity 220-aa GST protein; precise epitope not determined

Species/Isotype Mouse monoclonal IgG_1

Cross-reactivity Negligible with bacterial, yeast, insect, or mammalian cell lysates

Sensitivity 2.5-5 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with chemiluminescent substrates)

Applications Immunoblotting, immunoprecipitation, and immunolocalization

Form Stablized solution in 50% glycerol

Working dilution 1:10,000 for immunoblotting

Product	Size	Cat. No.
GST•Tag™ Monoclonal Antibody	50 μg	71097-3
	250 μg	71097-4

His•Tag® Monoclonal Antibody

Sensitive, specific detection of His•Tag fusion proteins

Specificity HisHisHisHisHis; N-terminal, C-terminal or internal

Species/Isotype Mouse monoclonal IgG_1

Cross-reactivity Negligible with bacterial, yeast, insect, or mammalian cell lysates

Sensitivity 2 ng (Western blot developed with chromogenic substrates)

Applications Immunoblotting, immunoprecipitation, and immunolocalization

Form Lyophilized, BSA-free

Working dilution 1:1000-1:2000 of antibody working solution [lyophilized antibody should be dissolved in 15 μl (3 μg) or 500 μl (100 μg) sterile water prior to dilution]

Product	Size	Cat. No.
His•Tag® Monoclonal Antibody	100 μg	70796-3
	3 μg	70796-4

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