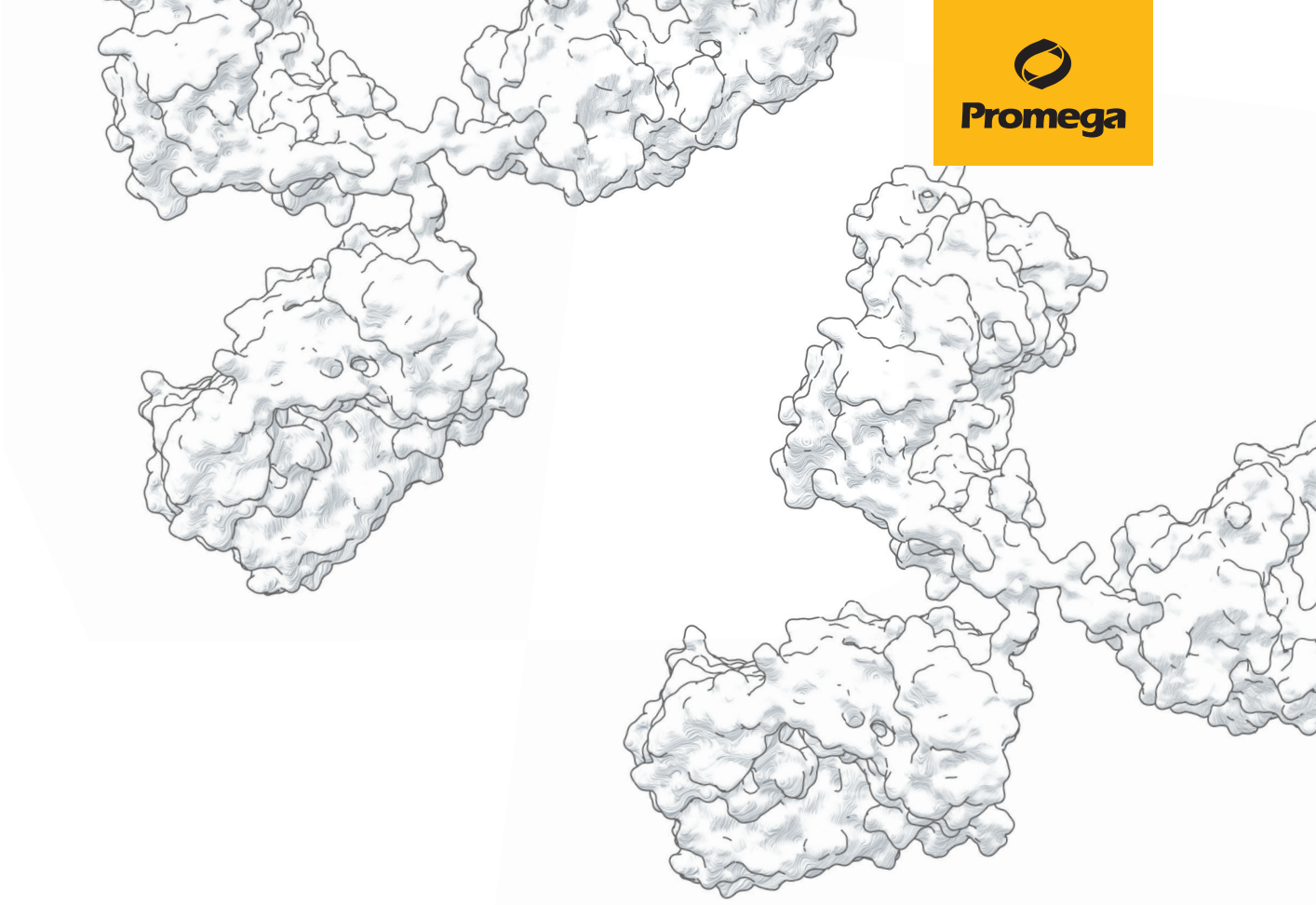
8 Protein Detection Tools for Western Blotting & ELISA



Protein Detection Tools for Western Blotting and ELISA

Western blot and ELISA are widely used analytical techniques for the specific detection of proteins in samples such as cells, tissues and other extracts. For both techniques protein-specific antibodies (primary antibodies) are required. Upon binding of a primary antibody to its target, a conjugated secondary antibody directed against a species-specific heavy chain portion of the primary antibody is added: for example, an anti-mouse secondary antibody binds to a primary antibody generated in mouse. Secondary antibodies, which are most frequently conjugated to reporter enzymes such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), will bind to one primary antibody. Depending on the substrates used, the conjugated enzymes catalyze a colorimetric, fluorescent or chemiluminescent reaction enabling sensitive detection with different instruments and scanners.

Table 8.1. Overview of Substrates for Western Blotting and ELISA.

Readout	Secondary Antibody Conjugates	
	Alkaline Phosphatase	Horseradish Peroxidase
Luminescent	-	ECL Western Blotting Substrate[W] (Cat.# W1001, W1015)
Fluorescent	AttoPhos® AP Fluorescent Substrate[E] (Cat.# S1000)	-
Colorimetric	ProtoBlot® AP Systems (BCIP/NBT)[W] (Cat.# W3940, W3950, W3960) Western Blue® Stabilized Substrate[W] (Cat.# S3841)	TMB Stabilized Substrate[W] (Cat.# W4121) TMB One Solution[E] (Cat.# G7431)

[E] ELISA
[W] Western Blotting

Table 8.2. Overview of Promega Conjugated Secondary Antibodies.

Cat.#	Conjugated Secondary Antibodies	Size	Recommended Dilution	Storage	Applications*
Alkaline Phosphatase conjugates (AP)					
S3721	Goat Anti-Mouse IgG (H+L), AP Conjugate	100µl	1:7,500	+4°C	W, D, E
S3731	Goat Anti-Rabbit IgG (Fc), AP Conjugate	100µl	1:7,500	+4°C	W, D, E
S3821	Goat Anti-Human IgG (H+L), AP Conjugate	100µl	1:7,500	+4°C	W, D, E
S3831	Goat Anti-Rat IgG (H+L), AP Conjugate	100µl	1:2,500	+4°C	W, D, E
Horseradish Peroxidase conjugates (HRP)					
W4021	Goat Anti-Mouse IgG (H+L), HRP Conjugate	300µl	1:2,500	+4°C	W, D, E
W4011	Goat Anti-Rabbit IgG (H+L), HRP Conjugate	300µl	1:2,500	-20°C/+4°C	W, D, E
W4031	Goat Anti-Human IgG (H+L), HRP Conjugate	300µl	1:2,500	+4°C	W, D, E
G1351	Rabbit Anti-Chicken IgY, HRP Conjugate	300µl	1:1,000	–20/+4°C	W, D, E
Anti-ACTIVE® qualified antibodies					
V1151	Donkey Anti-Goat IgG, AP	60µl	1:5,000-10,000	-20°C	W
V7951	Donkey Anti-Rabbit IgG (H+L) HRP	60µl	1:5,000-10,000	-20°C/+4°C	W
V8051	Donkey Anti-Goat IgG, HRP	60µl	1:5,000-10,000	-20°C	W

*W: Western Blotting; D: Dot Blotting; E: ELISA

Conjugated Secondary Antibodies

Detection of primary antibodies in Western blotting, enzyme-linked immunosorbent assay (ELISA) and dot blotting.

Description

High-quality, polyclonal secondary antibodies are raised in goat, rabbit or donkey. These polyclonal antibodies are immunoaffinity-purified using corresponding immobilized antigens. They are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes.

The Anti-ACTIVE® qualified secondary antibodies are specifically tested for the use with Promega Anti-ACTIVE® primary antibodies, which are tools to measure activation of three members of the Mitogen-Activated Protein Kinase (MAPK) superfamily. The primary antibodies are specific for dually-phosphorylated active forms of MAPK, p38 and JNK. They exhibit minimal cross-reactivity to goat, mouse and sheep IgG, bovine serum albumin (BSA) and proteins in mammalian cell extracts. These secondary antibody conjugates provide low backgrounds and highly specific signals.

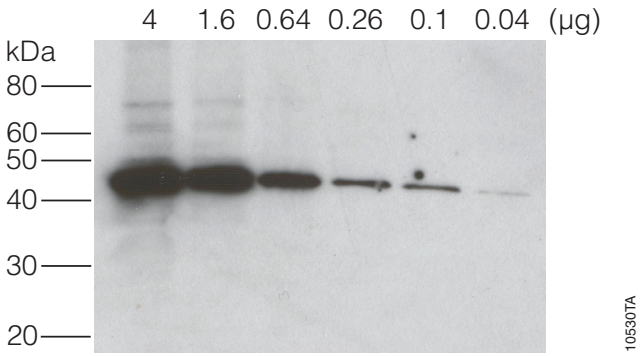


Figure 8.1. Western blot (immunoblot) for β-actin in cytoplasmic lysate from HEK293T cells. The blot was performed on a serial dilution of lysate; each lane contains an indicated amount of total protein (µg). Primary antibody (monoclonal) was used at 1:5,000; Anti-Mouse IgG, HRP Conjugate secondary antibody (Cat.# W4021) was used at 1:2,500; blot imaged with ECL Western Blotting Substrate (Cat.# W1015) and 1-minute exposure.

Features and Benefits

- **Approved:** Use with confidence, as supported by numerous publications.
- **Ready-to-Use Formulation:** No need to reconstitute the antibody.
- **Flexible Dispensing:** We can readily accommodate large-scale custom orders.

References

Chang, H.-Y. *et al.* (2011) Domain analysis of protein P30 in *Mycoplasma pneumoniae* cytadherence and gliding motility. *J. Bacteriol.* **193**(7), 1726–33.

Prenner, G. *et al.* (2011) Is LEAFY a useful marker gene for the flower-inflorescence boundary in the *Euphorbia cyathium*? *J. Exp. Bot.* **62**(1), 345–50.

Hu, D. *et al.* (2012) Novel insight into KLF4 proteolytic regulation in estrogen receptor signaling and breast carcinogenesis. *J. Biol. Chem*. **287**(17), 13584–97.

AttoPhos® AP Fluorescent Substrate System

Fluorescent development of ELISA.

Description

The AttoPhos® AP Fluorescent Substrate System provides a highly sensitive fluorescent alkaline phosphatase (AP) substrate. The system includes AttoPhos® Substrate, AttoPhos® Buffer and Calibration Solution. AttoPhos® Substrate is cleaved by alkaline phosphatase to produce inorganic phosphate (Pi) and the alcohol 2′-[2-benzothiazoyl]-6′-hydroxybenzothiazole (BBT).

This enzyme-catalyzed conversion of the phosphate form of AttoPhos® Substrate to BBT is accompanied by an enhancement in fluorescence properties. Relative to AttoPhos® Substrate, the BBT anion has highly increased quantum efficiency and fluorescence excitation, also the emission spectra are shifted into the visible region. Relative to other fluorometric reporters, the BBT anion has an unusually large Stokes' shift of 120nm, resulting in a higher signal-to-noise ratio and higher overall detection sensitivity. The excitation of the fluorescence is at 435nm, emission at 555nm.

Features and Benefits

- **Sensitivity:** Low fluorescence signal until enzymatically activated, detection of AP to 0.1 attomole.
- **Low Background:** Low fluorescence from interfering biological molecules.
- **Linearity:** Linear kinetics over five orders of magnitude of AP concentration.

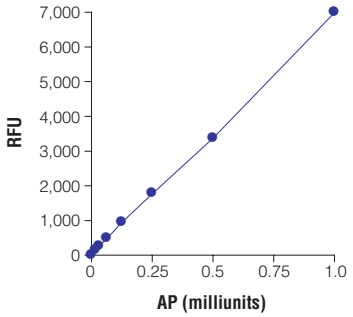


Figure 8.2. Fluorescence signal observed for a serial dilution of calf intestinal alkaline phosphatase (CIAP) treated with 1mM AttoPhos® Substrate in a multiwell plate. The graph represents the increasing fluorescence per unit of AP at 15 minutes post addition of AttoPhos® Substrate.

References

Michaud, A. *et al.* (2013) Absence of cell surface expression of human ACE leads to perinatal death. *Hum. Mol. Genet.* Nov. 14 [Epub ahead of print]

Meinel, S. *et al.* (2013) Mineralocorticoid receptor interaction with SP1 generates a new response element for pathophysiologically relevant gene expression. *Nucl. Acids Res.* **41**(17), 8045–60.

Chen, Y. *et al.* (2013) Common tolerance mechanisms, but distinct cross-reactivities associated with gp41 and lipids, limit production of HIV-1 broad neutralizing antibodies 2FS and 4E10. *J. Immunol.* **191**(3), 1260–75.

Ordering Information

AttoPhos® AP Fluorescent Substrate System
(Cat.# S1000)

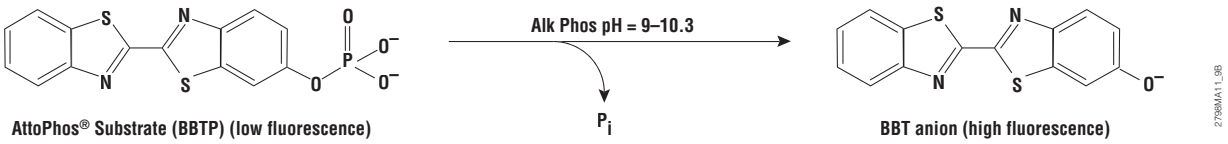


Figure 8.3. The reaction of AttoPhos® Substrate with AP to produce highly fluorescent BBT and inorganic phosphate (Pi).

TMB One Solution

Colorimetric Development of ELISA.

Description

TMB One Solution is a safe, convenient ready-to-use working solution for the detection of HRP-conjugated antibodies in an ELISA format. HRP-conjugated antibodies react with the chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (TMB) yielding a blue-colored solution. After reaching the desired color intensity, the reaction is stopped by addition of an acidic solution, which leads to a change in color from blue to yellow. Plates are analyzed on an ELISA reader at 450nm.

Features and Benefits

- **Convenient:** Single solution provided ready-to-use; add, incubate, stop and read. This homogeneous reagent reduces assay variation.
- **Stable:** Stable for 12 months at 4°C, providing extended shelf life; the assay end product is stable for at least one hour after stopping the assay.
- **Safe:** Provided in a slightly acidic, nonhazardous proprietary buffer without aprotic solvents; non-caustic to plastics used in automated systems.
- **Sensitive:** Low background provides higher assay sensitivity.

Additional Information

The TMB One Solution has been developed to work with Promega E_{max}® ELISAs for BDNF, GDNF, and TGFβ1. It can be used for any ELISA using HRP conjugated secondary antibodies.

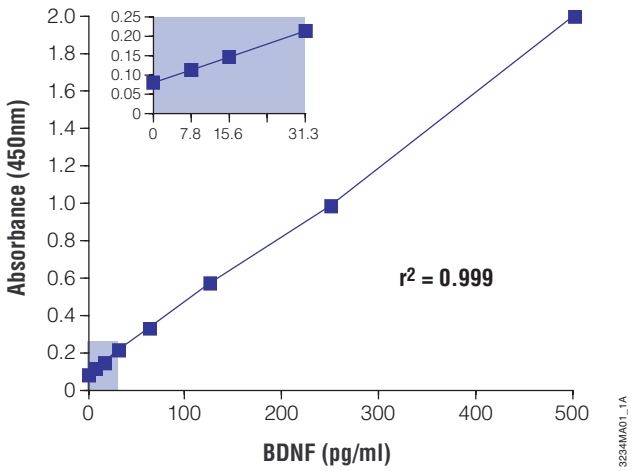


Figure 8.4. Representative BDNF standard curve obtained using the ELISA "BDNF E_{max}® ImmunoAssay System" (Cat.# G7610). The inset is an enlargement of the 0–31.3pg/ml portion of the graph.

References

Smith, A.D. *et al.* (2013) Selenium status alters the immune response and expulsion of adult *Heligosomoides bakeri* worms in mice. *Infect. Immun.* **81**, 2546–53.

Yamazaki, T. *et al.* (2012) The ddY mouse: a model of postprandial hypertriglyceridemia in response to dietary fat. *J. Lipid Res*. **53**, 2024–37.

Apidianakis, Y. *et al.* (2012) Down-regulation of glutathione S-transferase o 4 (hGSTA4) in the muscle of thermally injured patients is indicative of susceptibility to bacterial infection. *FASEB J.* **26**, 730–7.

Ordering Information

TMB One Solution (Cat.# G7431)

Western Blue® Stabilized Substrate for Alkaline Phosphatase (AP)

Substrate for Alkaline Phosphatase for Western blots and Dot blots.

Description

Western Blue® Stabilized Substrate for Alkaline Phosphatase is a stable, ready-to-use substrate for Western blots and immunoscreening. It is a mixture of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitro blue tetrazolium (NBT) in a proprietary stabilizing buffer. Western Blue® Substrate should be used directly and without dilution. This liquid substrate deposits a permanent dark purple stain on membrane at sites with an alkaline phosphatase-conjugated antibody. Western Blue® Substrate is as sensitive as other reagents based on the BCIP/NBT formulation.

Features and Benefits

- **Convenient:** Ready-to-use formulation does not require dilution or reagent mixing.
- **Sensitive:** Substrate is as sensitive as other commercially available BCIP/NBT formulations and reagents.
- **Stable:** Stable for one year at room temperature.

References

Cedeno-Laurent, F. *et al.* (2012) Galectin-1 triggers an immunoregulatory signature in Th cells functionally defined by IL-10 expression. *J. Immunol.* **188**(7), 3127–37.

Petrova, N.S. *et al.* (2012) Carrier-free cellular uptake and the gene-silencing activity of the lipophilic siRNAs is strongly affected by the length of the linker between siRNA and lipophilic group. *Nucl. Acids Res*. **40**(5), 2330–44.

To, W.S. and Midwood, K.S. (2011) Identification of novel and distinct binding sites within Tenascin-C for soluble and fibrillar fibronectin. *J. Biol. Chem*. **286**(17), 14881–91.

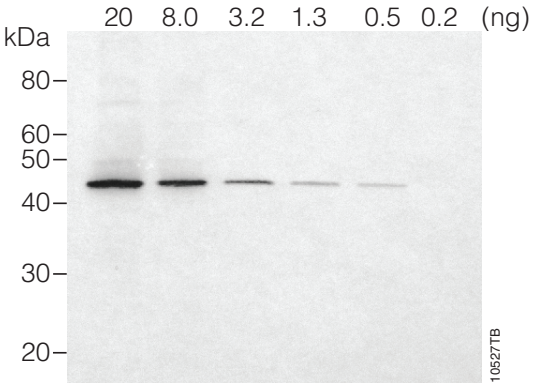


Figure 8.5. Western blot (immunoblot) for β-actin in cytoplasmic lysate from HEK293T cells. The blot was performed on a serial dilution of lysate; each lane contains an indicated amount of β-actin (ng). Primary antibody (monoclonal) used at 1:5,000; Anti-Mouse IgG, AP Conjugate secondary antibody (Cat.# S3721) used at 1:2,500; blot imaged with Western® Blue Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841).

Ordering Information

Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841)

TMB Stabilized Substrate for Horseradish Peroxidase (HRP)

Substrate for Western blots and Dot blots.

Description

TMB Stabilized Substrate for horseradish peroxidase is a stable, ready-to-use TMB (3,3´, 5,5´-tetramethyl-benzidine) color development substrate for localization of horseradish peroxidase-conjugated antibodies on Dot blots and Western blots. It is easier to use than 4-chloro-1-naphthol (CN), which must be prepared immediately before use. TMB Stabilized Substrate comes premixed and fully diluted in a proprietary buffer containing less than 0.5% organic solvent.

Features and Benefits

- **Convenient:** Premixed, ready-to-use; in proprietary buffer containing less than 0.5% organic solvents.
- **Stable:** Stable at room temperature for 12 months.
- **Sensitive:** At least 3X more sensitive than 4-chloro-1-naphthol (CN).
- **Long-Lasting Color:** Color is much more stable than CN and photographs more easily.

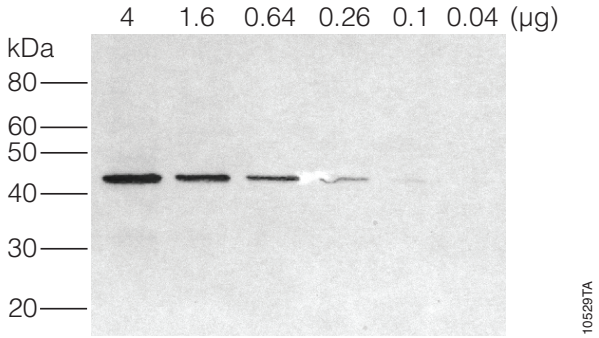


Figure 8.6. Western blot (immunoblot) for β-actin in cytoplasmic lysate from HEK293T cells. The blot was performed on a serial dilution of lysate; each lane contains an indicated amount of total protein (µg). Primary antibody (monoclonal) used at 1:5,000; Anti-Mouse IgG, HRP Conjugate secondary antibody (Cat.# W4021) used at 1:2,500; blot imaged with TMB Stabilized Substrate for horseradish peroxidase (Cat.# W4121).

Ordering Information

TMB Stabilized Substrate for HRP
(Cat.# W4121)

ECL Western Blotting Substrate

Substrate for the detection of HRP-conjugated antibodies for Western blots and Dot blots.

Description

ECL Western Blotting Substrate is a nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) conjugates on immunoblots. The ECL Western Blotting Substrate detects and visualizes the presence of picogram (pg) amounts of antigen through the use of photographic or other suitable chemiluminescent imaging methods.

Features and Benefits

- **Save Time:** No optimization required; you can switch from other entry-level ECL substrates.
- **Save Money:** Use Promega's Entry Level ECL.

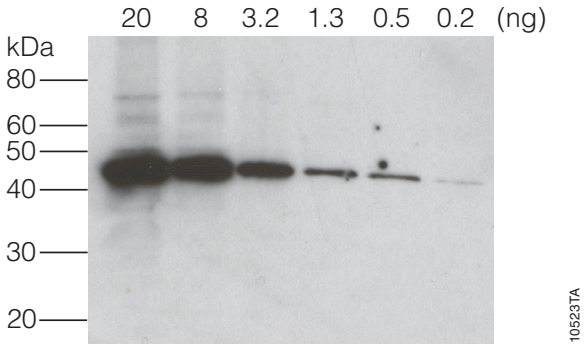


Figure 8.7. Western blot (immunoblot) for β-actin in cytoplasmic lysate from HEK293T cells. The blot was performed on a serial dilution of lysate; each lane contains an indicated amount of β-actin (ng) as quantitated by ELISA.

Ordering Information

ECL Western Blotting Substrate
(Cat.# **W1001, W1015**)

ProtoBlot® II AP Systems with Stabilized Substrate and Western Express® Fast Blotting

Substrate for the detection of alkaline phosphatase (AP) conjugated antibodies in Western blots.

Description and Principle

The ProtoBlot® II AP Systems with Stabilized Substrate are designed for the rapid and sensitive detection of proteins or other macromolecular antigens immobilized on nitrocellulose or PVDF membranes. Proteins can be transferred from gels after electrophoresis (Western blots) or bound directly from solution ("dot" blots).

The Western Express® Fast Blotting Protocol is included with the system and can reduce dramatically the time required to perform immunodetection.

Features and Benefits

- **Fast:** Easy-to-use Western Express® Protocol allows the detection of dot blots in 30–45 minutes and the detection of Western blots in 1–2 hours.
- **Convenient:** The system contains Western Blue® Stabilized Substrate for AP, which is a ready-to-use solution of BCIP/NBT. No reagent preparation is required for the substrate.

Ordering Information

ProtoBlot® II AP System with Stabilized Substrate, Human (Cat.# W3940)

ProtoBlot® II AP System with Stabilized Substrate, Mouse (Cat.# W3950)

ProtoBlot® II AP System with Stabilized Substrate, Rabbit (Cat.# W3960)

Broad Range Protein Molecular Weight Markers

SDS-PAGE protein size marker.

Description

The Broad Range Protein Molecular Weight Markers consist of nine clearly identifiable bands at convenient molecular weights. The protein sizes are 10, 15, 25, 35, 50, 75, 100, 150 and 225kDa. Each protein is present at a concentration of 0.1µg/µl, except for the 50kDa protein, which is present at 0.3µg/µl and serves as a reference indicator, having triple the band intensity of the other proteins.

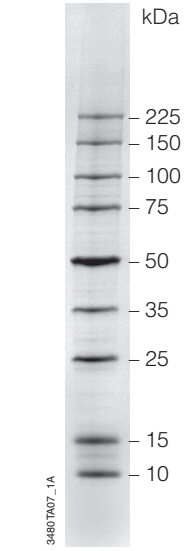
These markers are intended for use as a size standard when performing SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) for estimation of the molecular weight of the protein of interest. Note that they are not stained and will need to be visualized using common in-gel staining reagents such as Coomassie®, Silver, or other staining methods

Features and Benefits

- **Reference Band:** Band at 50kDa is 3X intensity for use as a reference.
- **Convenient:** Nine bands at evenly spaced intervals.
- **Fast:** Ready to load.

Additional Information

Sufficient for 100 lanes at 5µl per lane.



4–20% Tris-Glycine SDS-PAGE gel

Figure 8.8. Broad Range Protein Molecular Weight Markers; 4-20% Tris-Glycine SDS-PAGE.

Ordering Information

Broad Range Protein Molecular Weight Markers (Cat.# V8491)

Protease Inhibitor Cocktail

Inhibition of endogenous proteases during protein purification from mammalian or insect cell cultures.

Description

Protease Inhibitor Cocktail is used to prevent protein degradation after lysing cells. The product is a mixture of six different protease inhibitors with different target protease specificities. The inhibitor cocktail is EDTA-free and provided as a powder, ready for reconstitution in 1ml of either 100% ethanol or DMSO to obtain a 50X working solution.

Features and Benefits

- **Broad Specificity:** Inhibitor cocktail is effective against a diverse number of proteases.
- **Excellent Potency:** Reagent provides the best-in-class level of protease inhibition.
- **Highly Compatible:** Works with a wide array of protein fusion tags (e.g., Flag® tag, His tag, GST tag) and capture technologies. It is ideally suited for HaloTag® Technology-based approaches.

References

Galbraith, M.D. *et al.* (2013)HIF1A Employs CDK8-Mediator to Stimulate RNAPII Elongation in Response to Hypoxia. *Cell* **153**(6), 1327–1339.

Deplu, R. *et al.* (2013) TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J.* **32**(5), 645–55.

Additional Information

Table 8.3. Compounds included in the Protease Inhibitor Cocktail.

Inhibitor	Mode of Action	Target
Benzamidine HCl	Reversible	Trypsin like/Serine Proteases
Leupeptin	Reversible	Serine/Cysteine Proteases
Pepstatin A	Reversible	Aspartic Acid Proteases
1,10 Phenanthroline	Chelator	Metalloproteases
PMSF	Reversible	Serin Proteases
Bestatin	Reversible	Amino Peptidases

Ordering Information

Protease Inhibitor Cocktail (Cat.# G6521)