# Protein Detection Tools for Western Blotting & ELISA

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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.

<table>
<thead>
<tr>
<th>Readout</th>
<th>Secondary Antibody Conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Alkaline Phosphatase</strong></td>
</tr>
<tr>
<td><strong>Luminescent</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Fluorescent</strong></td>
<td>AttoPhos&lt;sup&gt;®&lt;/sup&gt; AP Fluorescent Substrate&lt;sup&gt;F&lt;/sup&gt; (Cat.# S1000)</td>
</tr>
<tr>
<td><strong>Colorimetric</strong></td>
<td>ProtoBlot&lt;sup&gt;®&lt;/sup&gt; AP Systems (BCIP/NBT)&lt;sup&gt;W&lt;/sup&gt; (Cat.# W3940, W3950, W3960)</td>
</tr>
<tr>
<td></td>
<td>Western Blue&lt;sup&gt;®&lt;/sup&gt; Stabilized Substrate&lt;sup&gt;W&lt;/sup&gt; (Cat.# S3841)</td>
</tr>
</tbody>
</table>

<sup>E</sup> ELISA  
<sup>W</sup> Western Blotting

### Table 8.2. Overview of Promega Conjugated Secondary Antibodies.

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

*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.

**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.

**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.

**References**


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](image1)

Figure 8.2. Fluorescence signal observed for a serial dilution of calf intestinal alkaline phosphatase (CIP) 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.

![Figure 8.3](image2)

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

References

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

References
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
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(8), 2330–44.
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’-tetramethylbenzidine) 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 photograph 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 $\beta$-actin in cytoplasmic lysate from HEK293T cells. The blot was performed on a serial dilution of lysate; each lane contains an indicated amount of $\beta$-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.

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

Additional Information

Table 8.3. Compounds included in the Protease Inhibitor Cocktail.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Mode of Action</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamidine HCl</td>
<td>Reversible</td>
<td>Trypsin like/Serine Proteases</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>Reversible</td>
<td>Serine/Cysteine Proteases</td>
</tr>
<tr>
<td>Pepstatin A</td>
<td>Reversible</td>
<td>Aspartic Acid Proteases</td>
</tr>
<tr>
<td>1,10 Phenanthroline</td>
<td>Chelator</td>
<td>Metalloproteases</td>
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<tr>
<td>PMSF</td>
<td>Reversible</td>
<td>Serine Proteases</td>
</tr>
<tr>
<td>Bestatin</td>
<td>Reversible</td>
<td>Amino Peptidases</td>
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</tbody>
</table>

References
