

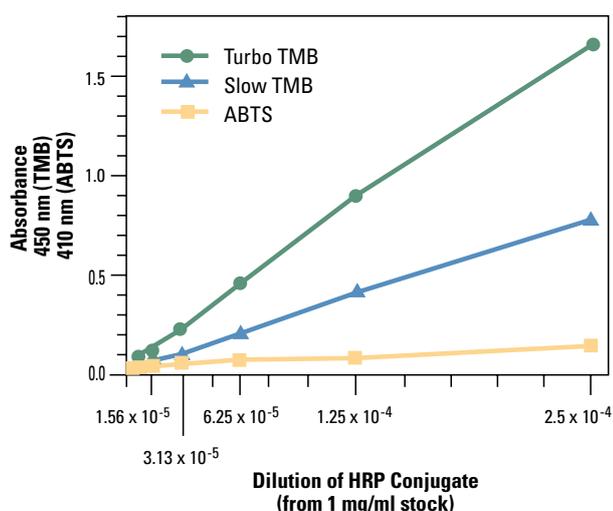
# Electrophoresis, Blotting and Immunodetection

## Enzyme substrates/kits - Colourimetric

### TMB substrate solutions and kit, Thermo Scientific Pierce

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Comparison of sensitivities of Thermo Scientific Pierce Turbo TMB, Slow TMB and ABT substrates.



TMB (3,3',5,5'-tetramethylbenzidine) is a chromogen that yields a blue colour (measurable at 370nm or 652nm) when oxidised with hydrogen peroxide (catalysed by HRP).

The colour then changes to yellow (measured at 450nm) upon addition of sulfuric or phosphoric acid to stop the reaction. TMB is very sensitive and more quickly oxidised than other HRP substrates, resulting in faster colour development. Thermo Scientific Pierce TMB substrate solutions are one-component substrates that require no preparation before use. Unlike other commercially available substrates, these products contain no DMF or DMSO.

There are three formulations that differ primarily in their sensitivities. Slow TMB is intermediate in sensitivity – ideal for kinetic readings. The sensitivity of the Turbo TMB compares to that of OPD used at approximately 1mg/mL. Ultra TMB-ELISA produces the highest signal:noise ratio and sensitivity in the picogram range.

Catalogue No	Description
PN34024	Slow TMB substrate solution, 250mL
PN34022	Turbo TMB substrate solution, 250mL
PN34028	Ultra TMB-ELISA substrate solution, 250mL
PN34021	TMB substrate kit. Includes: Peroxidase substrate (TMB), 200mL Peroxide solution (H <sub>2</sub> O <sub>2</sub> ), 200mL

### TMB-Blotting substrate solution, Thermo Scientific Pierce

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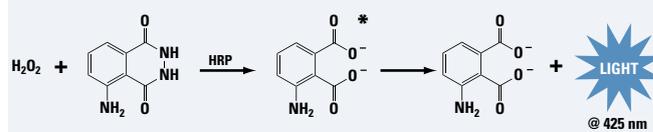
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Thermo Scientific Pierce TMB-Blotting is a single component peroxidase substrate for Western blotting and immunohistochemistry. Precipitating the product results in dark blue bands where the enzyme is located. Pierce TMB-Blotting is well suited to applications that require a high signal-to-noise ratio.

- Stable, pre-filtered and ready-to-use single component
- No hydrogen peroxide required
- Noncarcinogenic and no DMF or DMSO present
- Large dynamic range, high sensitivity, and minimal fading

Catalogue No	Description	Quantity, mL
PN34018	Pierce TMB-Blotting substrate solution	250

### Chemiluminescent substrates for Western blotting



**Chemiluminescence.** Luminol is oxidised in the presence of HRP and hydrogen peroxide to form an excited state product (3-aminophthalate). The 3-aminophthalate emits light at 425nm as it decays to the ground state.

When energy in the form of light is released from a substance because of a chemical reaction, the process is called chemiluminescence. Luminol is one of the most widely used chemiluminescent reagents, and its oxidation by peroxide results in creation of an excited state product called 3-aminophthalate. This product decays to a lower energy state by releasing photons of light. Enhanced chemiluminescence (ECL) is made possible with proprietary additives that greatly increase the intensity and duration (stability) of light emitted by the luminol reaction.

Chemiluminescent substrates have steadily gained in popularity because they offer several advantages over other detection methods. These advantages have allowed chemiluminescence to become the detection method of choice in most protein laboratories. Using chemiluminescence allows multiple exposures to obtain the best image. The detection reagents can be removed and the entire blot reprobed to visualise another protein or to optimise detection of the first protein. A large linear response range allows detection and quantitation for a large range of protein concentrations. Most importantly, chemiluminescence yields the greatest sensitivity of any available detection method.

Chemiluminescent substrates differ from other substrates in that the light detected is a transient product of the reaction that is only present while the enzyme-substrate reaction is occurring. This is in contrast to substrates that produce a stable, coloured product; these coloured precipitates remain on the membrane after the enzyme-substrate reaction has terminated. On a chemiluminescent Western blot, the substrate is the limiting reagent in the reaction; as it is exhausted, light production decreases and eventually ceases. A well-optimised procedure using the proper antibody dilutions will produce a stable output of light for several hours, allowing consistent and sensitive detection of proteins. When the antibody is not diluted sufficiently, a stable output of light will never be achieved. Too much enzyme in the system will rapidly oxidise the substrate and terminate the signal.

### ECL Western blotting substrate, Thermo Scientific Pierce

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A reliable ECL formulation

- No optimisation required - you can switch to our ECL substrate without the need for optimisation or protocol changes

Thermo Scientific Pierce ECL Western blotting substrate is an entry level Western blotting substrate.

Catalogue No	Description
PN32106	Pierce ECL Western blotting substrate, 500mL kit Sufficient substrate for 4,000cm <sup>2</sup> of membrane
PN32209	Pierce ECL Western blotting substrate, 250mL kit Sufficient substrate for 2,000cm <sup>2</sup> of membrane
PN32109	Pierce ECL Western blotting substrate, 50mL kit Sufficient substrate for 400cm <sup>2</sup> of membrane