

Guide to enzyme substrates for western blotting

Enzyme substrates for western blotting are available in a range of sensitivities for different detection methods. Select a technology and sensitivity to match your experimental requirements.

Chemiluminescent western blotting substrates

Horseradish peroxidase (HRP) substrates

Thermo Scientific™ substrate	Cat. No.	Signal duration	Detection limit	Primary and secondary antibody dilutions (from 1 mg/mL stock)	Features
SuperSignal™ West Atto Ultimate Sensitivity Substrate	A38554 (20 mL)	6 hours	Low femtogram to high attogram	1°: 1:5,000 2°: 1:100,000 to 1:250,000	Highest sensitivity substrate for very low-abundance proteins, limited samples, or limited antibodies
	A38555 (100 mL)				
	A38556 (200 mL)				
SuperSignal™ West Femto Maximum Sensitivity Substrate	34094 (20 mL)	8 hours	Low to mid femtogram	1°: 1:5,000 2°: 1:100,000 to 1:500,000	High sensitivity substrate for low-abundance proteins
	34095 (100 mL)				
	34096 (200 mL)				
SuperSignal™ West Dura Extended Duration Substrate	37071 (20 mL)	24 hours	Mid femtogram	1°: 1:5,000 2°: 1:50,000 to 1:250,000	Long duration substrate ideal for quantitation
	34075 (100 mL)				
	34076 (200 mL)				
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	34579 (20 mL)	Up to 24 hours	Low picogram to high femtogram	1°: 1:5,000 2°: 1:20,000 to 1:100,000	Wide dynamic range for everyday, routine western blotting applications
	34577 (200 mL)				
	34580 (500 mL)				
	34578 (1 L)				
Pierce™ ECL Plus Western Blotting Substrate	32134 (25 mL)	5 hours	Low picogram to mid femtogram	1°: 1:1,000 2°: 1:25,000 to 1:200,000	Can be detected via chemiluminescent and fluorescent imaging technologies; absorption and emission maxima of the substrate are 430 nm and 503 nm, respectively
	32132 (100 mL)				
Pierce™ ECL Western Blotting Substrate	32109 (50 mL)	1–2 hours	Low picogram	1°: 1:1,000 2°: 1:5,000 to 1:10,000	Low cost substrate for high-abundance protein targets
	32209 (250 mL)				
	32106 (500 mL)				

Alkaline phosphatase (AP) substrates

Invitrogen™ substrate	Cat. No.	Recommended membrane	Detection limit	Primary and secondary antibody dilutions (from 1 mg/mL stock)	Maximum light levels reached
Novex™ AP Chemiluminescent Substrate (CDP-Star™)	WP20002 (100 mL)	PVDF, nylon	Low to mid femtogram	1°: 1:500 to 1:5,000 2°: 1:2,000 to 1:10,000	1 second to 15 minutes
CDP-Star™ Substrate with Nitro-Block-II™ Enhancer	T2218 (100 mL)	Nitrocellulose	Low to mid femtogram	1°: 1:500 to 1:5,000 2°: 1:2,000 to 1:10,000	1 second to 15 minutes
CSPD™ Substrate	T2141 (50 mL)	PVDF, nylon	High picogram	1°: 1:500 to 1:5,000 2°: 1:2,000 to 1:10,000	2 minutes to 2 hours
	T2142 (100 mL)				
	T2143 (250 mL)				
CSPD™ Substrate with Nitro-Block™ Enhancer	T2217 (100 mL)	Nitrocellulose, PVDF	High picogram	1°: 1:500 to 5,000 2°: 1:2,000 to 1:10,000	2 minutes to 2 hours

Colorimetric western blotting substrates (precipitating substrates)

HRP substrates

Thermo Scientific™ substrate	Cat. No.	Color	Detection limit	Primary and secondary antibody dilutions (from 1 mg/mL stock)	Features
Metal Enhanced DAB Substrate Kit	34065 (275 mL)	Brown–black	17 pg	1°: 1:1,000 2°: 1:5,000 to 1:50,000	2-component reagent kit
1-Step™ Ultra TMB Blotting Solution	37574 (250 mL)	Dark blue	20 pg	1°: 1:1,000 2°: 1:5,000 to 1:10,000	Ready-to-use solution
Pierce™ CN/DAB Substrate Kit	34000 (275 mL)	Black	500 pg	1°: 1:500 2°: 1:2,000 to 1:20,000	2-component reagent kit
Pierce™ DAB Substrate Kit	34002 (275 mL)	Brown	1 ng	1°: 1:500 2°: 1:2,000 to 1:20,000	2-component reagent kit
1-Step™ TMB-Blotting Substrate Solution	34018 (250 mL)	Dark blue	1 ng	1°: 1:500 2°: 1:2,000 to 1:20,000	Ready-to-use solution
1-Step™ Chloronaphthol Substrate Solution	34012 (250 mL)	Blue–purple	5 ng	1°: 1:500 2°: 1:2,000 to 1:20,000	Ready-to-use solution

AP substrates

Thermo Scientific™ substrate	Cat. No.	Color	Detection limit	Primary and secondary antibody dilutions (from 1 mg/mL stock)	Features
1-Step™ NBT/BCIP Substrate Solution	34042 (250 mL)	Black–purple	30 pg	1°: 1:500 2°: 1:5,000 to 1:50,000	Ready-to-use solution

Find more information at thermofisher.com/detect