Thermo SCIENTIFIC

Guide to enzyme substrates for ELISA

TR0033.4

Thermo Scientific Pierce Enzyme Substrates for immunoassays are available in a range of sensitivities for different detection methods. Certain substrates will perform better than others when optimized for a given system and detection limit. While optimization is required to get the best results from any assay system, the following guide is provided as a starting point for both choosing an appropriate substrate and the initial primary and secondary antibody dilutions.

Colorimetric ELISA substrates. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) substrates for use with an absorbance plate reader listed in order of increasing sensitivity.

Enzyme Conjugate	Thermo Scientific Product	Product #	Total Assays[1]	Absorbance Color[3]	Detection Limit[2]	1° / 2° Ab dilution[2] (from 1 mg/ml stock)
AP	1-Step PNPP	37621	1,000 wells	405 nm Yellow	10 ng/well [100 ng/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
AP	PNPP, Phosphatase Substrate Kit	37620	5,250 wells	405 nm Yellow	10 ng/well [100 ng/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
AP	PNPP, 105 tablets	34047	5,250 wells	405 nm Yellow	10 ng/well [100 ng/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
AP	PNPP, 25 g	34045	250,000 wells	405 nm Yellow	10 ng/well [100 ng/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
HRP	1-Step ABTS	37615	2,000 wells	410 nm (650 nm) Green	250 pg/well [2.5 ng/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	ABTS, 50 tablets	34026	3,330 wells	410 nm (650 nm) Green	250 pg/well [2.5 ng/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	OPD, 25 g	34005	5,000 wells	490 nm (450 nm) Green (Orange)	7 pg/well [70 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	OPD, 50 tablets	34006	5,000 wells	490 nm (450 nm) Green (Orange)	7 pg/well [70 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	1-Step Slow TMB	34024	2,500 wells	450 nm (652 nm) Yellow (Blue)	8 pg/well [80 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	1-Step Turbo TMB	34022	2,500 wells	450 nm (652 nm) Yellow (Blue)	7 pg/well [70 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	TMB Substrate Kit	34021	4,000 wells	450 nm (652 nm) Yellow (Blue)	6 pg/well [60 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000
HRP	1-Step Ultra TMB	34028	2,500 wells	450 nm (652 nm) Yellow (Blue)	2 pg/well [20 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:50,000

[1] Value is the number of wells in a 96-well microplate that can be processed. For additional information about required materials and assay considerations that determine the number of assays that may be performed, please see the product instructions.

[2] Detection limits and recommended antibody dilutions have been generalized as a means to begin optimization. Individual assays may require conditions outside the ranges suggested here.

[3] For substrates that have a change in maximum absorbance wavelength and color after the reaction is stopped, the values are indicated in parenthesis.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | http://www.thermoscientific.com/pierce

Chemiluminescent ELISA substrates. Horseradish peroxidase (HRP) substrates for use with a luminometer or other plate reader capable of measuring total luminescence using no ilters and excitation wavelength listed in order of increasing sensitivity.

Enzyme Conjugate	Thermo Scientific Product	Product #	Total Assays[1]	Emax Color[3]	Detection Limit[2]	1° / 2° Ab dilution[2] (from 1 mg/ml stock)
HRP	SuperSignal® ELISA Pico	37070	1,000 wells	425 nm Blue/Green	500 fg/well [5 pg/ml]	1° 1: 1,000 2° 1: 10,000 - 1:50,000
HRP	SuperSignal ELISA Pico	37069	2,500 wells	425 nm Blue/Green	500 fg/well [5 pg/ml]	1° 1: 1,000 2° 1: 10,000 - 1:50,000
HRP	SuperSignal ELISA Femto	37075	1,000 wells	425 nm Blue/Green	170 fg/well [1.7 pg/ml]	1° 1: 1,000 2° 1: 50,000 - 1:100,000
HRP	SuperSignal ELISA Femto	37074	2,500 wells	425 nm Blue/Green	170 fg/well [1.7 pg/ml]	1° 1: 1,000 2° 1: 50,000 - 1:100,000

[1] Value is the number of wells in a 96-well microplate that can be processed. For additional information about required materials and assay considerations that determine the number of assays that may be performed, please see the product instructions.

[2] Detection limits and recommended antibody dilutions have been generalized as a means to begin optimization. Individual assays may require conditions outside the ranges suggested here.

[3] The peak emission wavelength is given for reference. However, for best sensitivity, measure total light output using a luminometer.

Chemifluorescent ELISA substrates. Horseradish peroxidase (HRP). Substrates for use with a fluorescent plate reader listed in order of increasing sensitivity.

Enzyme Conjugate	Thermo Scientific Product	Product #	Total Assays[1]	Emax (Em) Amax (Ex)	Detection Limit[2]	1° / 2° Ab dilution[2] (from 1 mg/ml stock)
HRP	QuantaBlu® Fluorogenic Substrate	15169	2,700 wells	420 nm 325 nm	500 fg/well [5 pg/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
HRP	QuantaBlu Kinetic Fluorogenic Substrate	15162	2,700 wells	420 nm 325 nm	500 fg/well [5 pg/ml]	1° 1: 500 2° 1: 5,000 - 1:20,000
HRP	QuantaRed® Enhanced Fluorescent Substrate	15159	1,000 wells	585 nm 570 nm	400 fg/well [4 pg/ml]	1° 1: 1,000 2° 1: 5,000 - 1:20,000

[1] Value is the number of wells in a 96-well microplate that can be processed. For additional information about required materials and assay considerations that determine the number of assays that may be performed, please see the product instructions.

[2] Detection limits and recommended antibody dilutions have been generalized as a means to begin optimization. Individual assays may require conditions outside the ranges suggested here.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.