

TMB Substrate Set

Catalog# / Size	421101 / 110 ml x 2
Regulatory Status	RUO
Other Names	TMB Substrate
Description	The TMB Substrate Set contains 110 ml TMB Substrate A and 110 ml TMB Substrate B, sufficient for twenty 96-well plates. It is formulated with 3, 3', 5, 5' tetramethyl benzidine (TMB). The substrate can be catalyzed with peroxidase to produce a pale blue color which can be read spectrophotometrically at 370 or 620-650 nm. The TMB reaction may be stopped with BioLegend's Stop Solution (Cat. No. 423001), and read at 450 nm.

Product Details

Storage & Handling	The TMB Substrate Set (TMB Substrate A and TMB Substrate B) should be stored between 2°C and 8°C. Avoid prolonged exposure to light, contact with metal, air, or extreme temperatures.
Application	ELISA
Recommended Usage	TMB Substrate A should be clear and colorless. TMB Substrate B should be colorless to a very light amber. TMB Substrate is the mixture of equal volumes of TMB Substrate A with TMB Substrate B. For example, for one plate, mix 5.5 ml TMB Substrate A with 5.5 ml of TMB Substrate B in a clean container. Mix immediately before use. After mixing the reagents together, TMB substrate working solution should be colorless or very faint blue.
Application Notes	Warm to room temperature prior to use. To prepare a working solution, mix equal volumes of TMB Substrate A and TMB Substrate B. Prepare only the amount needed for each assay run. After wells are completely washed, to remove unbound antibody and HRP, add 100 µl to 200 µl substrate solution to each well. Discard any remaining working solution after use. For endpoint assays, add 50 µl to 100 µl of Stop Solution (2N H ₂ SO ₄ or 1M H ₃ PO ₄), which changes the color from blue to yellow. Read at 450 nm (minus 570 nm for wavelength correction) within 30 minutes of stopping the reaction. For kinetic assays, read the blue color at 620 - 650 nm.
Application References	<ol style="list-style-type: none"> Kim MS, <i>et al.</i> 2008. <i>J Biochem.</i> 143:497. PubMed McNally A, <i>et al.</i> 2011. <i>PNAS.</i> 108:7529. PubMed Santiago FW, <i>et al.</i> 2012. <i>Vaccine.</i> 30:4606. PubMed Wan LY, <i>et al.</i> 2014. <i>Toxicol Sci.</i> PubMed de Pablo P, <i>et al.</i> 2014. <i>Ann Rheum Dis.</i> 73:580. PubMed
(PubMed link indicates BioLegend citation)	
Product Citations	<ol style="list-style-type: none"> Santiago F, <i>et al.</i> 2012. <i>Vaccine.</i> 30:4606. PubMed Wan L, <i>et al.</i> 2014. <i>Toxicol Sci.</i> 139:83. PubMed Pablo P, <i>et al.</i> 2014. <i>Ann Rheum Dis.</i> 73:580. PubMed Yang E, <i>et al.</i> 2015. <i>Acta Biochim Biophys Sin.</i> 47: 588-596. PubMed Parrish H, <i>et al.</i> 2016. <i>Proc Natl Acad Sci U S A.</i> 113: 3000 - 3005. PubMed Morgan S, <i>et al.</i> 2016. <i>J Immunol.</i> 196: 5014 - 5023. PubMed Liu W, <i>et al.</i> 2016. <i>J Virol.</i> 90: 8496 - 8508. PubMed Uematsu T, <i>et al.</i> 2016. <i>Sci Rep.</i> 6:37815. PubMed Nogales A, <i>et al.</i> 2017. <i>Virology.</i> 500:1-10. PubMed Surawut S, <i>et al.</i> 2017. <i>Sci Rep.</i> 7:40006. PubMed Salehi S, <i>et al.</i> 2017. <i>PLoS One.</i> 10.1371/journal.pone.0163614. PubMed Nuvolone M, <i>et al.</i> 2017. <i>PLoS One.</i> 12:e0171923. PubMed Debernardi S, <i>et al.</i> 2020. <i>PLoS Med.</i> 17:e1003489. PubMed Qi T, <i>et al.</i> 2020. <i>Cell Rep.</i> 33:108452. PubMed Leppkes M, <i>et al.</i> 2020. <i>EBioMedicine.</i> 58:102925. PubMed Malik V, <i>et al.</i> 2020. <i>Int J Mol Sci.</i> 21:00. PubMed Groeneveld D, <i>et al.</i> 2020. <i>J Hepatol.</i> 72:146. PubMed Walczewska M, <i>et al.</i> 2017. <i>Adv Exp Med Biol.</i> 10.1007/978-94-024-1079-2_41. PubMed Del Alcazar D, <i>et al.</i> 2019. <i>Cell Rep.</i> 28:3047. PubMed Pagan JD, <i>et al.</i> 2018. <i>Cell.</i> 172:564. PubMed Oh E, <i>et al.</i> 2018. <i>Front Oncol.</i> 0.716666667. PubMed Stebegg M, <i>et al.</i> 2019. <i>Nat Commun.</i> 2.113194444. PubMed

23. Garg S, *et al.* 2019. *Biotechniques*. 67:166. [PubMed](#)
24. Vanderleyden I, *et al.* 2020. *Cell Rep*. 30:611. [PubMed](#)
25. Schwaderer J, *et al.* 2020. *Cell Death Dis*. 11:154. [PubMed](#)
26. Kim M, *et al.* 2008. *J Biochem*. 143:497. [PubMed](#)
27. McNally A, *et al.* 2011. *Proc Natl Acad Sci U S A*. 108:7529. [PubMed](#)

Antigen Details

Gene ID NA

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