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Product Data Sheet

TMB Substrate Set

Catalog # / Size: 421101 / 110 ml x 2

Storage: 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.

Applications:

Applications: 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_2SO_4 or 1M H_3PO_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:
1. Kim MS, *et al.* 2008. *J Biochem.* 143:497. PubMed
2. McNally A, *et al.* 2011. *PNAS.* 108:7529. PubMed
3. Santiago FW, *et al.* 2012. *Vaccine.* 30:4606. PubMed
4. Wan LY, *et al.* 2014. *Toxicol Sci.* PubMed
5. de Pablo P, *et al.* 2014. *Ann Rheum Dis.* 73:580. PubMed

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.

Related Products:

Product	Clone	Application
HRP Streptavidin		ELISA, ELISPOT, IHC, WB
HRP Avidin		ELISA, ELISPOT, IHC, WB
ELISA Assay Diluent (5X)		ELISA
Stop Solution for TMB Substrate		ELISA

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