

## This product is for research use only (not for diagnostic or therapeutic use)

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## Product no AS15 Diluent AgriseraTMB Diluent (100 ml)

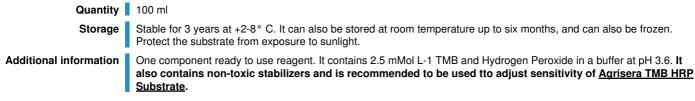
## Product information

3,3',5,5'-Tetramethylbenzidine, horseradish peroxidase (AS15 TMB-HRP) is a sensitive reagent that is ready to use for the quantitative detection of HRP bound to a solid phase or in free solution. It is designed to detect and quantify specific peptide/protein/hormone in a complex mixture in enzyme linked immunosorbent assay (ELISA). It utilizes the enzymatic reaction triggered by horse raddish peroxidase (HRP) in which a chromogenic, chemiluminiescent or chemifluorescent substrates are oxidized resulting in color change, chemiluminiscence or chemifluorescence respectively.

A one-electron oxidation product is formed in the presence of HRP and hydrogen peroxide. This is a cation free radical, it is blue in color with adsorption maximum at 653 nm. The a reaction with HRP, hydrogen peroxide or acidification of the radical with acid yields the diimine terminal oxidation product that adsorbs light at 450 nm. The extinction coefficient of the radical ( $E653 \text{ nm} = 3.9 \times 104 \text{ mol-1 cm-1}$ ) and diimine ( $E450 \text{ nm} = 5.9 \times 104 \text{ mol-1 cm-1}$ ) provides a very sensitive system for this assay.

After incubations with antibodies or HRP labeled reagents, TMB solution is added. As an alternative TMB can also be spiked with a different volume of buffered HRP to create dilution series of this substrate, for example 50 %, 70%. This will change sensitivity of this product and can make it more suitable for certain assays, depending what kinetics are required. If you require to adjust sensitivity of your assay, please check Agrisera TMB/Diluent pack.

The oxidation of TMB by HRP produces a blue reaction product, measured at 650 nm. Color formation can be recorded as a function of time or the reaction can be stopped using an equal volume of 0.3 M sulfuric acid after a fixed interval. Increased sensitivity is achieved by converting the blue radical to the yellow diimine by addition of acid. The resulting yellow chromogen is measured immediately at 450 nm.



## **Application information**