

**Product no** **AS15 TMB-HRP****AgriseraTMB HRP Substrate****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, chemiluminescent or chemifluorescent substrates are oxidized resulting in color change, chemiluminescence 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 (  $E_{653\text{ nm}} = 3.9 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$  ) and diimine (  $E_{450\text{ nm}} = 5.9 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$  ) 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.

**Storage** | Stable for 3 years at +2-8° C or at room temperature up to six months, and can also be frozen. Storage at room temperature for 1 month results in ca, 2 % activity loss. Protect the substrate from exposure to sunlight.

**Additional information** | One component ready to use reagent. It contains 2.5 mMol L-1 TMB and Hydrogen Peroxide in a buffer at pH 3.6. It also contains non-toxic stabilizers.

**How to work with this reagent?**

- Pour out amount of reagent which is needed for your assay.
- Do not our back any unused substrate back to the original bottle.
- Any trace metals, HRP or other contaminant may be reason for the substrate to turn blue.

**Application information**