

### ***Product Specification/Description Sheet***

es(HS)TMB, Cat. ID/No. #esTMB

#### **Description:**

**es(HS)TMB**, enhanced soluble (High Sensitivity) TMB Substrate for ELISA (#esTMB), is a peroxidase substrate containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in an aqueous buffer formulation. This product is supplied as a one component ready-to-use reagent. Intact substrate is clear colorless to slightly yellowish in appearance. Reaction with peroxidase results in converting substrate into soluble blue (~ 650 nm) product that after stopping with strong acid further turns to super-oxidized soluble yellow product. Yellow product can be measured at 450 nm (measuring at 450 nm vs. 620-650 nm reference wavelength will additionally improve readout patterns in ELISA).

**es(HS)TMB** is optimal for ~15-min. substrate development reaction in ELISA. Shaker mode will improve overall performance. **es(HS)TMB** is equally well applicable in protocols with longer, up to ~30 minutes, substrate reaction times.

**es(HS)TMB** substrate yields exceptionally high signals with zero background and therefore is particularly recommended for using in Ultra-Sensitive ELISA tests. Compared to our s(HS)TMB that was already and is still one of the strongest TMB based ELISA substrates, **es(HS)TMB** gives on the average 30% larger signals by the same clean backgrounds.

It does not contain high-density components, is not viscous, and has no detergent or colloidal activity. It does not foam and does not "stick" to hydrophobic surfaces. This allows easy and precise dispensing: standard PP and HD-PE pipette tips repel **es(HS)TMB** almost restlessly, as it were pure water.

**es(HS)TMB** features capacity of spontaneously recovering to the colorless state: if it has turned bluish on light, it will become again completely colorless after several hours keeping it in dark. **es(HS)TMB** endures at least 4 recovery cycles: colorless → light → bluish → dark → colorless, etc. without drop in activity.

After stopping, fixed reaction remains stable during several following hours, also under direct light. There is no fading, neither increase in color. This concerns both low signals (blank, background, negative control) and high positive signals - no precipitation in wells with OD up to 3.500. Stable readout picture makes later postponed measuring in ELISA-spectrophotometer unproblematic and improves robustness when working with **es(HS)TMB** in larger multi-plate ELISA series. This, along with allowing flexible reaction times, makes it ideal for HS blood bank screening applications.

#### **Stability, Storage and Handling:**

**es(HS)TMB** is stable two years at +2°C/+8°C or one year at ambient room temperature (18°C/28°C).

Both storage regimens are equally applicable depending on desired duration of storage. Within first year, storage in refrigerator will neither improve, nor worsen product performance. +4°C is however a preferred storage regimen providing better safety against possible adverse influence of higher temperatures. **es(HS)TMB** tolerates at least one freezing event showing after thawing no change in appearance and performance.

Warm up **es(HS)TMB** substrate, if stored at +4°C, to assay (room) temperature prior to use.  
Avoid exposure to direct sunlight, air and extreme temperatures.

For repackaging bulk product use only clean intact amber Nunc/Nalge(n) or Wheaton HD-PE bottles. Bottle substrate in light-protected (weak diffused light) and clean area gravimetrically relying on  $d^{20^{\circ}\text{C}} \approx 1 \text{ g/ml}$ . Alternatively use peristaltic pump with certified compatible tubing (e.g. Norprene or Cole-Parmer/Millipore C-Flex tubing). Do not re-filter. For dispensing smaller volumes, use close-design syringe dispensers (e.g. CombiTips of Eppendorf).

#### **Safety and Hazard Identification:**

**es(HS)TMB** substrate does not contain volatile or other organic solvents and is non caustic and non corrosive to plastic, glass and metal materials used in automatic dispensing and robotic ELISA instrumentation. It is a completely water based product added with non-toxic proprietary stabilizers and enhances that are not health-hazardous. This product is non-hazardous, non-toxic, non-carcinogenic, environmentally safe unrestricted product.