

TMB

Peroxidase Substrate

Cat. No.	SK-4400
Storage	Store reagents in original bottles at 2-8 °C. Avoid storing reagents or working solution in strong direct light.
Description	This kit contains all of the reagents necessary to prepare the working solution. The TMB Substrate (3, 3', 5, 5'-tetramethylbenzidine) produces a blue reaction product in the presence of peroxidase (HRP) enzyme. On tissue sections, TMB is more sensitive than other peroxidase substrates but the reaction product has a more diffuse appearance. By omitting the TMB Reagent 3 (stabilizing solution) from the TMB kit the working solution will produce a soluble blue product useful for ELISA.

Components

Product Name	Volume
TMB Reagent 1	6 ml
TMB Reagent 2	6 ml
TMB Reagent 3	6 ml
TMB Reagent 4	6 ml

Preparation of Substrate Working Solution

To 5.0 ml of distilled water

- Add 2 drops ($\approx 72 \mu\text{l}^\dagger$) of TMB Reagent 1
- Add 3 drops ($\approx 81 \mu\text{l}^\dagger$) of TMB Reagent 2
- Add 2 drops ($\approx 80 \mu\text{l}^\dagger$) of TMB Reagent 3
- Add 2 drops ($\approx 80 \mu\text{l}^\dagger$) of the TMB Reagent 4

Mix well before use. Use immediately.

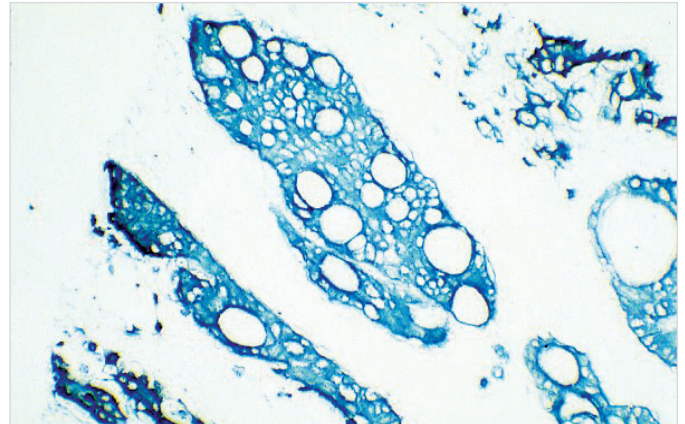
† Drop volumes differ due to solvent compositions.

IMPORTANT: Little is known about the toxicity and carcinogenicity of the substrate kit components. Appropriate care should be exercised when using this reagent including gloves, eye protection, lab coats, and good laboratory procedures. Dispose in accordance with local regulations.

Instructions for Use

For Tissues or Cells:

After incubation with a peroxidase (HRP) detection system, rinse sections in buffer. Incubate with the substrate working solution for 5-15 minutes. Optimal development times should be determined by the investigator.



Tumor: Cytokeratin (s) detected with VECTASTAIN® Elite® ABC Kit and TMB Substrate (blue). No counterstain.

Wash slides with buffer for 2-3 minutes. Rinse in water and counterstain if desired. Excessive wash times after substrate reaction can cause decreased sensitivity.

For permanent, non-aqueous mounting: Dehydrate, clear, and coverslip using a non-aqueous mounting media, such as VectaMount® Mounting Medium (H-5000) or VectaMount® Express Mounting Medium (H-5700). Do not mount with aqueous mounting media.

Use in ELISA:

To use the TMB Substrate for ELISA, do not add the TMB Reagent 3 (stabilizing solution). Measure the absorbance of the soluble blue product at 650 nm. To increase absorbance of the blue the TMB product two to four-fold, stop the reaction by adding 50 μl of 1N sulfuric acid per microtiter plate well. Measure the absorbance of the yellow reaction product at 450 nm.

Notes

We recommend using glass-distilled water in the preparation of the substrate buffer. Deionized water may contain inhibitors of the peroxidase reaction.

Detailed product listings, specifications, protocols and additional information is available on our website: vectorlabs.com