

PRODUCT INFORMATION

Product name: TMB solution
Cat. No.: GM61
Size: 500 ml
Store at: -20°C

Introduction:

3,3',5,5'-Tetramethylbenzidine or TMB is the most popular chromogenic substrate for horseradish peroxidase (HRP). TMB is used in immunohistochemistry as well as being a visualising reagent used in enzyme-linked immunosorbent assays (ELISA). In presence of HRP, the reaction produces a deep blue byproduct, which is then analysed on a spectrophotometer at a wavelength of 370 or 655 nm. TMB solution is optimized for use with either nitrocellulose or PVDF Western blotted membranes.

Components:

50 ml of TMB Substrate (1 mg/ml) in DMSO

450 ml of Substrate Dilution Buffer

Materials to be prepared by customer:

30% hydrogen peroxide

Protocol for membrane staining:

1. Remove unbound antibody and HRP.
2. Warm TMB Substrate and Substrate Buffer to room temperature prior to use.
3. Prepare the solution by adding **1 ml TMB Substrate to 9 ml Substrate Dilution Buffer, and then add 2 µl of fresh 30% hydrogen peroxide**. Vortex and use immediately. This should be operated in the dark.
4. At dark place, transfer the above mixture (from step 3) into the container with the membrane (NC or PVDF), incubate at 37°C with rotation for about 5 - 15 minutes, or until the desired color is achieved. (incubation times may vary depending on your assay). A blue band should appear on membrane.
5. Once the desired color is achieved, stop the reaction by immersing membrane in reagent quality water for 20 - 30 seconds. The reaction should be stopped before background color becomes too intense resulting in insufficient contrast between positive staining and background.
6. Take photo and analyze the result.

Note:

1. The incubation time for reaction should not exceed 30 minutes.
2. A 6x8 cm membrane is recommended. For larger membrane, increase the volume of solution accordingly, so that the gel is completely immersed.
3. Freshly prepare the solution prior to use.
4. TMB solution may develop a yellow tinge over time; this does not affect product performance.

Storage:

Can be stored at 4°C for 6 months ; -20°C for 24 months.



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