



TMB SUBSTRATE KIT FOR PEROXIDASE

Catalog Number SK-4400

The TMB substrate kit contains all of the reagents necessary to prepare a working solution of 3, 3', 5, 5'-tetramethylbenzidine (TMB) for staining nitrocellulose, nylon, other membranes, and tissue sections. TMB will yield a blue precipitate. On tissue sections, TMB reaction product has a more diffuse appearance than other peroxidase substrates but is significantly more sensitive. By deleting the Stabilizing Solution (*Step 3), the TMB kit will produce a soluble blue product useful for enzyme immunoassays.

DISPENSING REAGENTS:

For convenience the reagents are supplied in dropper bottles. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the opaque caps on the bottles when they are not in use. **DO NOT PIPET REAGENTS DIRECTLY FROM BOTTLES.** Drop volumes of each component may be different due to solvent characteristics. Proper concentrations of substrate components are assured in preparing the working solutions by using the drop dispensers only.

INSTRUCTIONS FOR USE:

Use in Membrane Staining

Immediately before use, prepare the substrate solution as follows:

1. To 5.0 ml of distilled water, add 2 drops of the Buffer Stock Solution and mix well.
2. Add 3 drops of the TMB Stock Solution and mix well.
- *3. Add 2 drops of the Stabilizing Solution and mix well.
4. Add 2 drops of the Hydrogen Peroxide Solution and mix well.

Transfer membrane to a different staining dish (which has not been exposed to peroxidase), containing TMB substrate solution sufficient to completely cover the membrane. Development time is generally 10-30 minutes at room temperature. When development is satisfactory, wash membrane briefly in distilled or deionized water. Air dry. (Note: Excessive washing may cause a loss of colored precipitate from the membrane.) When using larger membranes, the water volume in Step 1 may be increased to 15 ml.

Use in Immunohistochemical Staining

Incubate tissue sections with the substrate solution (prepared as listed above) at room temperature until suitable staining develops. Ten minutes generally provides good stain intensity. Excessive wash times after substrate reaction can cause decreased sensitivity. Wash sections in buffer for 2-3 minutes, rinse briefly in water, dehydrate, clear, and air dry sections prior to permanently mounting (using *VectaMount™*, Cat. No. H-5000) and coverslipping. To avoid loss of sensitivity, once coverslipped do not dry slides in a 56 °C oven. Aqueous mounting may cause fading of substrate reaction product. TMB substrate is compatible with Vector® Nuclear Fast Red Counterstain (Cat. No. H-3403). After counterstaining, do not wash section more than 5 minutes to avoid reduction in sensitivity. (See reverse for counterstain compatibility chart.)

Use in Enzyme Immunoassays

To use the TMB substrate for enzyme immunoassays, do not add the Stabilizing Solution (*Step 3). Measure the absorbance of the soluble blue product at 650 nm. To increase absorbance of the blue 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.

The reagents should be stored at 4 °C and protected from light when not in use.

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.