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# Product Information

**TMB Enhanced One Component HRP Membrane Substrate**

Catalog Number **T9455**
Storage Temperature 2-8 °C

## TECHNICAL BULLETIN

### Product Description

TMB Enhanced One Component HRP Membrane Substrate is a ready to use solution containing 3,3',5,5'-tetramethylbenzidine in a mildly acidic buffer. The substrate reacts with peroxidase to form an insoluble permanent dark blue/purple color on the membrane or surface to which it is applied. This enhanced formulation extends the dynamic range of detection, especially at higher concentrations of peroxidase where other substrates may exhibit loss of signal due to washout effects. This substrate is not recommended for microwell or immunohistochemical applications. Unreacted substrate should be faint yellow to pink in appearance.

### Available Package Sizes

| Catalog Number |
|---|
| T9455-100ML |
| T9455-1L |

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

TMB Enhanced One Component HRP Membrane Substrate is stable for 18 months from date of manufacture when stored at 2-25 °C. Storage is recommended at 2-8 °C. This product is stable for 4 years at 2-8° C. This substrate is light sensitive and should be protected from direct sunlight and UV sources. Over time, some flocculent material may appear; however, this does not affect product performance.

### Procedure

TMB Enhanced One Component HRP Membrane Substrate is supplied as a ready to use solution. The product should be allowed to equilibrate to room temperature (25 °C) prior to use. For use, completely cover the membrane surface with substrate. The substrate system will react with sites on the membrane containing peroxidase, producing an insoluble permanent dark blue reaction product. To stop the reaction, rinse the membrane with reagent quality water. The reaction should be monitored and read before the background color becomes too intense which may result in diminished contrast between the positive and background staining. Dilution of the substrate is not recommended. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted.

<u>ImmunoBlot Application:</u>

1. Equilibrate TMB Enhanced One Component HRP Membrane Substrate (T9455) to room temperature if stored at 2-8 °C.
2. Transfer proteins to membrane as per your established protocol.
3. Remove membrane from the transfer apparatus and place in blocking buffer for 30–60 minutes at room temperature or overnight at 4° C.
4. Remove blocking solution, add primary antibody to the membrane, and incubate for 30–60 minutes at room temperature or overnight at 4° C.
5. If the primary antibody is directly conjugated to HRP, proceed to step 7.
6. If using secondary antibody, wash the membrane 3X with either TBS or PBS containing 0.05% TWEEN-20®. Remove the wash solution and incubate with secondary antibody for 30–60 minutes at room temperature or overnight at 4° C.

7. Wash the membrane 3X with either TBS or PBS containing 0.05% TWEEN-20.
8. Remove all solution and carefully blot off excess with paper towel.
9. Add enough room temperature TMB Enhanced One Component HRP Membrane Substrate to cover the entire membrane; monitor the reaction time carefully. Full color should develop within 10-30 minutes, but timing should be optimized for each assay. To stop the reaction, wash the membrane at least 2X in distilled water.

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