

**DIRECTIONS FOR USE**

Code E888
TMB Membrane Substrate
for use with peroxidase-labeled detection systems

**Contents:**

TMB Substrate Solution (Code E865)
TMB Membrane Buffer (Code E862)
Mixing/Dispensing Bottle

After mixing, these solutions yield sufficient substrate to detect 500 cm² of membrane (Sample Kits detect 50 cm²).

- Step 1** For each 50 cm² of membrane, aliquot 9 ml of TMB Substrate Solution and 1 ml of TMB Membrane Buffer into the Mixing/Dispensing Bottle. Cap tightly with included dropper cap and invert gently to mix.
- Step 2** Using the dropper cap, carefully layer the prepared substrate solution onto the membrane to be detected. The unused prepared substrate can be stored at 4°C (refrigerated) for up to 24 hours, without any loss of activity.
- Step 3** Develop for 5 minutes at room temperature, or until desired color development is obtained. Stop the reaction by rinsing for 5 seconds in deionized water. Any blue background seen will fade upon drying.
- Step 4** For best results, store developed membranes at room temperature, out of direct light.
- Step 5** For larger or smaller membranes, use 1 ml of TMB Substrate Solution and 100 µl TMB Membrane Buffer for each 5 cm² of membrane.



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