

**ANTIGENIX AMERICA Inc.**  
**1-800-558-1008**  
**PRODUCT INFORMATION SUMMARY**

**TMB Substrate Solutions:** cat no: ES200  
**Solutions Part A and Part B** 100 mL (2)

**(ELISA Construction Pack- component)**

**DESCRIPTION:**

TMB ( 3,3', 5,5' -Tetramethyl-Benzidine) substrate solution is used for ELISA applications in immunologic and serologic procedures where HRP (Horseradish Peroxidase) is applied. It is non-carcinogenic.

**HANDLING AND STORAGE:**

**PRODUCT USE:**

**Provided as 100 mL Solutions Part A and Part B.**

**Store DARK at 4 Deg. C.**

Prepare substrate solution no more than **15 minutes before last incubation of assay**: Mix one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated - DO NOT USE**. Use mixed substrate solution WITHIN 2 HOURS, AND AVOID DIRECT LIGHT.

Apply TMB solution to wells of assay as substrate. For 96-well microplates, usually 100 uL per well is recommended. Be sure the ELISA plate is **thoroughly washed ( 5 times)** before adding TMB solution. (Wash steps are critical!)

For most ELISA applications, incubate TMB substrate solution for 15-20 minutes at 37 Deg. C. Then add 100 uL stop solution (2N Sulphuric Acid) **and mix by gently tapping plate**, then read O.D. at 450 nm within 30 minutes. - or follow customer's usual protocol.

**CAUTION:**Substrate solution A contains 20% acetone - **flammable** - keep away from sources of heat or flame.