

# Stock Solution



TD-S Revision 2.0

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## TMB Dihydrochloride Stock Solution

[TMB dihydrochloride \(GoldBio Catalog # TMBHCL\)](#) is soluble in an acetic acid: water mixture (1:1 v/v), but is more commonly dissolved in phosphate citrate buffer. In 0.05M phosphate citrate buffer (pH 5.0) TMB dihydrochloride has a solubility of 0.1 mg/ml when heated at 45°C and sonicated.

### Instructions

Preparation of 0.05M Phosphate-Citrate Buffer

1. Add 25.7 ml of 0.2M dibasic sodium phosphate and 24.3 ml of 0.1M citric acid to 50 ml of molecular biology grade water.
2. Adjust pH to 5.0 if needed.

Solution of TMB in Phosphate-Citrate Buffer

1. Dissolve 1 mg of TMB dihydrochloride in 10 ml of 0.05M phosphate-citrate buffer (pH 5.0; preparation outlined above).
2. Immediately prior to use add 2  $\mu$ l of 30% hydrogen peroxide to every 10 ml of solution used.

**Note:** Before the addition of hydrogen peroxide the solution will remain colorless if tightly sealed and stored at 4°C, but if the solution is exposed to air oxidation will occur. If oxidation has occurred the solution will have a faint blue color.

### References

Liem, H.H., et al., (1979). Quantitative determination of hemoglobin and cytochemical staining for peroxidase using 3,3',5,5'-tetramethylbenzidine dihydrochloride, a safe substitute for benzidine. *Analytical Biochemistry*, 98(2), 388-393.

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