

Heme Stain

1. Dissolve 30 mg TMB (*N, N, N', N'*-tetramethylbenzidine) in 15ml MeOH. (Make sure it dissolved well before next step)
2. Add 35 ml of 0.5 M Na Acetate (pH 5.0)
3. Carefully place gel in stain
4. Wait for 3-5 min
5. Add 300 ul of H₂O₂ and mix
6. Wait for 5 min
7. Check for blue bands

Notes:

- Load 50 ug of total protein/well
- Keep the reaction in the dark all the time. TMB is light sensitive.

References:

- Francis Jr., R. T. a. B., R. R.** 1984. Specific indication of hemoproteins in polyacrylamide gels using a double-staining process. *Analytical Biochemistry* **136**:509-514.
- Thomas, P. E., Ryan, D. and Levin, W.** 1976. An Improved Staining procedure for the detection of the peroxidase activity of cytochrome p-450 on sodium dodecyl sulfate polyacrylamide gels. *Analytical Biochemistry* **75**:168-176.