

Heme stain protocol

Materials

- TMB (*N, N, N', N'*-tetramethylbenzidine)
- MeOH
- 0.5 M Na Acetate, pH 5.0
- H₂O₂

Method

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.