Buffers and Solutions (Prepare freshly)

2 M NaOH

Component	Final conc.	For 100 mL
10 M NaOH	2 M	20 mL
Dissolve in		100 mL with water

0.1 M HCl

Component	Final conc.	For 60 mL
5 M HCl	0.1 M	1.2 mL
Dissolve in		60 mL with water

Fe2/3 solution

Component	Final conc.	For 60 mL
FeCl3 (VWR #470301-382){target="_blank"}	0.333 M	3.24 g
FeCl2 · 4 H2O (VWR #470301-356){target="_blank"}	0.167 M	2 g
Dissolve in	0.1 M HCl	60 mL

Other materials:

- Ethanol
- Tetraethyl orthosilicate (Sigma #86578-250ML){target="_blank"}
- Ammonia solution (Sigma #1054321011){target="_blank"}

Procedure

1. Degas 100 mL 2 M NaOH and 60 mL Fe2/3 solution using a 0.22 µm filter.
2. Heat 100 mL 2 M NaOH in a 250 mL flat-bottom boiling flask with a stir bar at 100 rpm, 80˚C using a magnetic stirrer.
3. Use a dripping funnel to add 60 mL Fe2/3 solution dropwise into 100 mL 80˚C 2 M NaOH with stirring at 400 rpm. Alternatively, slowly pour 60 mL Fe2/3 solution into 100 mL 80˚C 2 M NaOH along the edge.

   !!! info "Note" * **Black precipitate of Fe3O4 (FeO·Fe2O3) is formed**

   ```
   * **Less oxygen, more yield**
   ```

4. Turn off heating and add 10 mL 25% ammonia solution, stir at 400 rpm for 30 min, and cool down to room temperature.
5. Transfer the solution to a plastic container and settle the magnetic particles using a strong magnet{target="_blank"}, discard the supernatant.
6. Resuspend in 200 mL EtOH, pellet magnetically, and discard the supernatant. Repeat twice.
7. Resuspend the pellet in 200 mL EtOH.
8. Mix 1.8 L of 99% EtOH with 50 mL of 25% ammonia solution and 200 mL of the synthesized iron oxide MNPs (from step 7) in a heat-resistant 5 L bottle using a magnetic stirrer (400 rpm). Switch on the heating and allow the solution to heat up to 80 °C.

   !!! info "Note" * **Make sure the iron oxide MNPs evenly distributed in the solution, prevent larget clusters**
9. Add 45 mL Tetraethyl orthosilicate (TEOS) under constant stirring and incubate for another 30 minutes.
10. Add 400 mL of ddH2O to the solution.
11. Allow the reaction to proceed overnight.
12. Cool down the solution to room temperature.
13. Separate the coated MNPs using a strong neodymium magnet.
14. Wash twice with pure water.
15. Wash twice with pure ethanol.
16. Resuspend the pellet with water to 50 mL.

    !!! info "Note" * **Generate ~ 15-20 mL magnetic beads each reaction** * **The magnetic beads should be pure black**

Reference

1. https://doi.org/10.1371/journal.pbio.3000107
2. https://bomb.bio/protocols/