

Iron-loading transferrin

Materials:

- apo-transferrin (iron-free transferrin; available from a number of commercial sources)
- PBS
- 1 mg/ml ferric ammonium citrate solution in 10 mM NaHCO₃ / 20 mM HEPES acid, pH 7.7
- PD-10 column (pre-packed Sephadex G-25 columns; Pharmacia Biotech) or any other desalting column
- 0.2 µm sterile syringe filter

1. Dissolve 200 mg apo-transferrin (iron-free) in 6 ml PBS.
2. Add 4 ml ferric ammonium citrate solution. (*The solution will be an ochre color.*)
3. Incubate at 37°C for 10 min. (*The solution will turn dark rust color.*)
4. During this incubation, equilibrate five prepacked PD-10 columns with PBS. (*Any desalting column can be used.*)
5. Run 2 ml iron-loaded transferrin over each column.
6. Collect the orange product (iron-loaded transferrin). Pool the orange fractions from each column.
7. Measure the OD of the sample at 280 and 465 nm. (The OD₂₈₀/OD₄₆₅ ratio should be 0.0496 if 100% of the transferrin is loaded with iron.)
8. Filter sterilize the iron-loaded transferrin with a 0.2 filter and store at 4°C.