## Overview and Considerations:

Gel filtration (size exclusion chromatography) separates biomolecules by size. The optimal separating range of Sephadex G-50 is 1500Da to 30,000Da, this means that proteins >30,000Da in size will elute in the void volume and that small proteins will be effectively separated from small salts. In this protocol, the volumes have been carefully calibrated and we typically recover proteins >10,000Da in size.

Related Techniques: G50 spin columns/Plates, PD10 columns

## Reagents:

1. Sephadex G-50 slurry (GE Healthcare)
2. OGT desalting buffer (see recipe)
3. Protein samples
4. Glass wool
5. Ice
6. 1-ml tuberculin syringe
7. 1.5-ml tubes, prechilled

## Protocol:

1. Start by placing a small amount of glass wool in the tip of the syringe (by tip, I mean the narrow part of the syringe that connects to the needle).
2. Pack a column containing exactly 1 ml of Sephadex G-50 slurry in a 1-ml tuberculin  
   syringe. To ensure that no bubble develop in the column bed, fill the syringe with water first.
   * Sephadex G-50 is usually supplied in 20% (v/v) ethanol. If not, the resin can be swelled in 20% (v/v) ethanol for several hours. We keep a stock of swolled G50 on the White Bench.
3. Wash the column with 5 ml desalting buffer to equilibrate the resin.
   * You can store the column at this point with a marble on top and a yellow cap on the bottom.
   * Before proceeding to the next step place the needle back on the syringe, and wash with 200ul of desalting buffer.
   * Note: When the needle is on the syringe, it is possible to run the column dry. This will lead to inconsistencies in the column bed, and disrupt the chromatography.
4. Load the protein sample onto the column. The volume of the sample can be up to 200 μl.
5. Wash the column with desalting buffer so that the total volume of this wash and the protein sample is 350 μl. For example:
   * If the sample volume is 150 μl, add 200 μl desalting buffer to the column at this step.
   * If the sample volume is 200 μl, add 150 μl desalting buffer to the column at this step.
6. Transfer the syringe column to a clean, prechilled 1.5-ml tube. Elute the protein with 200 μl desalting buffer. Keep on ice.
7. This is the desalted sample.