

Desalting

Desalting proteins/cell extracts using PD10 columns

Protocol:

Overview and Considerations:

1. Amersham Biosciences PD-10 Desalting columns are prepacked, disposable columns containing Sephadex™ G-25 Medium for group separation of high ($M_r > 5000$) from low molecular weight substances ($M_r < 1000$) by desalting and buffer exchange. Columns are delivered in a package which can be converted into a convenient
2. PD 10 columns are kept in the deli-box

Reagents:

1. PD-10 columns
2. Chromatography rack
3. Desired buffer

Protocol:

1. Cut the bottom off the PD10 column
2. Remove the cap and tip off the storage liquid
3. Place the column in the rack
4. Equilibrate the column with 25mls of buffer
5. Apply your sample in up to 2.5mls
 - If you have a 2ml sample, apply this first
 - Once this has run into the column, add the remaining 0.5mls
 - Discard flow through
6. Elute sample in 3.5mls, collect flow through as this is your desalted sample