

# Antibody Conjugation Kit Procedure Diagram

## Conjugation total time 4-5 Hrs.

A solution of antibody at 1 mg/ml in 300  $\mu$ l of PBS should be prepared. If antibody is at a concentration of 0.5 mg/mL concentrate 600  $\mu$ l to a final volume of 300  $\mu$ l to 1 mg/mL using standard concentrators spinning at 3200xG for 2 minutes.

Allow the reduction to proceed for 30 minutes. This should be coordinated to be completed just prior to the completion of the nanocrystal activation reaction.

While the antibody reduction and nanocrystal activation reactions proceed, equilibrate 2 de-salting columns with exchange buffer. Coordinate the timing such that the antibody desalting columns is completed just prior to the nanocrystal de-salting.

Materials collected from desalting columns may be collected separately for simplicity, then combined. Advanced users, however, may choose to collect the purified nanocrystals directly into the centrifuge tube used previously to collect the antibody materials.

Allow the conjugation reaction to proceed for 1 hr at room temperature.

Quench the conjugation reaction with 2-mercaptoethanol for 30 minutes at room temperature.

Equally partition the resultant solution (~1 mL) from the quench into 2 ultrafiltration devices. Concentrate to 10-20  $\mu$ l per tube. (Centrifuge at 7000 RPM for 10-15 min)

Load the concentrated solution from both ultrafiltration devices onto the separation media.

**NOTE: Collect no more than 10 drops from the column. Subsequent drops may contain significant concentrations of unconjugated antibody, which may interfere with subsequent assays.**

Nanocrystal activation requires 1 hr reaction time, using SMCC solution that has been pre-warmed to 37°C for 15 min to ensure complete dissolution of the SMCC.

**NOTE: Collect no more than 500-600 $\mu$ l from the antibody column and ~400-500 $\mu$ l from the nanocrystal column (total volume not to exceed 1 mL). Collection of additional material from either column may contaminate desired components with unreacted materials, causing undesirable results**

