



## Azo-Alpha-Cellulose and Azo-Avicel

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### PREPARATION:

Azo-Alpha Cellulose and Azo-Avicel are prepared by dyeing either alpha-cellulose or Avicel with Remazolbrilliant Blue dye to a dye content of approximately one dye molecule per 20 sugar residues.

Alpha-cellulose is an amorphous alkali-resistant cellulose. Avicel is a very organised (in a crystalline sense) microcrystalline cellulose.

### USES:

Azo-Alpha Cellulose and Azo-Avicel are both potential substrates for the assay of *endo*-1,4- $\beta$ -D-glucanase (*endo*-cellulase) and should give some indication of the effectiveness of cellulases in hydrolysing insoluble cellulose. It might be expected that the Azo-Avicel, being highly crystalline, would be more resistant to hydrolysis.

Megazyme does not offer standard curves relating the activity of particular cellulases on these substrates to release of water soluble dyed products, as the conditions of use may differ widely.

### POTENTIAL ASSAY FORMAT:

Incubate enzyme solution (0.2 mL) with a slurry of the substrate (1-2% w/v) in 100 mM sodium acetate buffer (pH 4.5) at 40°C for 1 hour. Terminate the reaction by adding 5 mL of 2 % trizma base solution (pH ~ 8.5) and centrifuge the slurry at 3,000 rpm in a bench centrifuge for 10 min. Carefully remove the supernatant solution and measure the absorbance at 590 nm.

For more accurate measurement of *endo*-1,4- $\beta$ -D-glucanase (*endo*-cellulase) activity, we recommend the use of the Megazyme Cellazyme C, or Beta-Glucazyme Test Tablets, or of Azo-CM-Cellulose (soluble substrate).