CONCANAVALIN A COATED SURFACE

TECHNICAL NOTE N. 15

General procedure for binding a biomolecule to Concanavalin A coated surface

1. dilute biomolecule (sample) to 0.5-5 μg/ml in an appropriate neutral pH buffer (Buffer should contain 1mM Ca^{++} and 1mM Mn^{++}; in fact these ions promote the interaction between saccharide groups and Concanavalin A coated surface)
2. proceed with incubation: conditions depend on biomolecule structure
3. wash four times to remove the unbound material
4. proceed with your specific test:
   - to point out the bound biomolecule
   - to use the bound biomolecule to point out a specific counter molecule

Example of test: binding specificity of Concanavalin A coated plates

1. Dilute AHIgG-HRP from 100 ng/ml to 12.5 ng/ml in pure distilled water containing 1 mM $CaCl_2 \cdot 2\ H_2O$ + 1 mM $MnCl_2 \cdot 4\ H_2O$
2. Add 100μl of each solution to the wells of Concanavalin A coated plate and incubate 30' R.T. Add the same solutions to albumin coated plate as comparison for evaluate the specificity of binding
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 μl /well of TMB substrate solution and incubate 10 minutes at room temperature
6. Stop the substrate reaction by adding 100 μl of sulphuric acid 1 N and read the optical density values at 450 nm