CONCANAVALIN A COATED SURFACE

Concanavalin A, belonging to the lectins family, is a Hemagglutinin from the common jack bean *Canavalia ensiformis*. It is well known that lectins have been used extensively for the isolation of glyco-conjugates and glycoproteins with specific carbohydrate structures.
Concanavalin A shows specific affinity for molecules containing α-D-mannopyranosyl, α-D-glucopyrannosyl and sterically related.
Concanavalin A coated surfaces offer a powerful and sensitive instrument for binding in specific way the carbohydrate fraction of glycoproteins, enzymes and cell membranes.
The optical properties of polystyrene remain unchanged, allowing to use the modified surface as powerful tool for diagnostic assays.
Example of applications:

- **interaction with glycoproteins, glycopeptides and enzyme-antibody conjugates**
- **polysaccharides and glycolipids**
- **interaction with cellular membranes, hormones and hormone receptors**

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