Affinidex™

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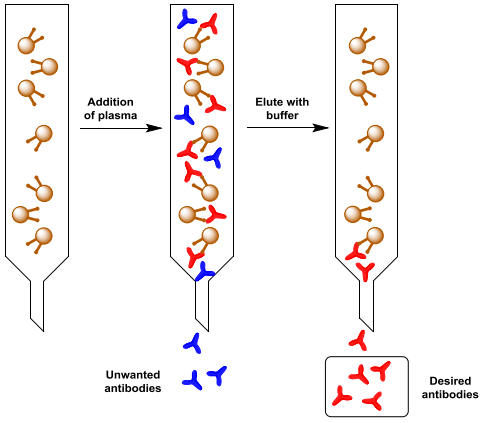
AFF101, AFFINIDEX™-A FF
Blood group A trisaccharide Sepharose-FF
AFF102, AFFINIDEX™-B FF
Blood group B trisaccharide Sepharose-FF

AFF201, AFFINIDEX™-A 4B
Blood group A trisaccharide Sepharose-4B
AFF202, AFFINIDEX™-B 4B
Blood group B trisaccharide Sepharose-4B



Affinidex™ Overview

The AFFINIDEX™ columns comprise our HAEMODEX™ A and B trisaccharides linked to Sepharose FF or Sepharose 4B for use as affinity matrices in research applications. These Sepahrose matrices are used routinely for experimental and developmental purposes.

The oligosaccharides are covalently linked to the polysaccharide gel via a 10-atom spacer to overcome steric hindrance. A loading of 0.6 µmole/mL is generally found to be sufficient, with little benefit seen from higher loadings.

AFFINIDEX™ columns can be used to:

- Remove anti-A and anti-B antibodies
- Study polymorphism
- Measure ligand density by lectin binding
- Clean up serum samples
- Purify antibodies

Assay Overview

The assay method detailed in this information sheet uses a coupled lectin-biotin, β-galactosidase-avidin enzyme assay to measure the binding of blood group oligosaccharides to a solid surface such as a Sepharose gel matrix or polymer beads.

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Materials

Quantities required are based on analysis of Blood group A and Blood group B oligosaccharides coupled to Sepharose gel matrix or polymer beads plus their corresponding control samples. The quantities outlined below will be enough to analyse a total of 4 samples.

Phosphate Buffered Saline Solution Dulbecco's PBS Buffer	280 mL
Helix pomatia lectin-biotin (Blood group A) Sigma, L6512	0.5 mg
Bandeiraea simplicifolia BS1-B4 lectin-biotin (Blood group B) Sigma, L2140	0.5 mg
Avidin-β-galactosidase complex Vector, A2300	2.0 mg
Sodiujm carbonate (1.0 M solution in water)	2.0 mL
2-Mercaptoethanol (1.0 M solution in water)	80 µL
2-Nitrophenyl-β-D-galactopyranose Orthonitrophenyl galactosidase (OPNG)	1.6 mL

The following solutions are best prepared directly prior to performing the assay:
Lectin-Biotin complex - 0.5 mg in 1 mL PBS
Avidin-β-galactosidase complex - 2 mg in 1 mL PBS
ONPG - 4 mg in 1 mL PBS (agitation/sonication required to aid dissolution)

Example preparations of the other solutions are:
1.0 M Sodium carbonate solution - 1.059 g in 10 mL water
1.0 M Mercaptoethanol - 700 µL in 10 mL water

Blank Preparation

1. Using a Gilson Pipette transfer 2.5 mL PBS, 20 µL mercaptoethanol solution and 200 µL OPNG solution to a vial.
2. Incubate at room temperature for 15 minutes.
3. Add a 500 µL aliquot of 1 M sodium carbonate buffer and mix.
4. Pipette the solution into a UV spectrophotomer cuvette.

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Sample Preparation

The Sepharose samples and controls should be prepared as follows:

1. Using a Gilson Pipette fitted with a wide bore tip transfer a 100 µL aliquot of the Sepharose gel solution/suspended beads to a 7 mL vial and dilute with 400 µL PBS.
2. Transfer 100 µL of the diluted Sepharose gel solution/suspended beads to a second 7 mL screw cap vial.
3. Add a 500 µL aliquot of the Lectin-Biotin complex (A or B as appropriate)
4. Incubate at room temperature for 30-45 minutes.
5. Set up the filtration apparatus with a 0.8 µm filter paper.
6. Pipette solution directly onto the 0.8 µm filter paper, rinsing the vial with 5x1 mL PBS.
7. Using a vacuum, slowly draw the solution through the filter paper, washing the gel/suspended beads solution with 3x10 mL PBS (*N.B. do not allow the beads to dry out as they will be difficult to resuspend*).
8. Re-suspend the gel in 2 mL PBS and add 100 µL of the Avidin-β-galactosidase complex, incubate in the filter apparatus for 3-4 minutes.
9. Using a vacuum, slowly draw the solution through the filter paper, washing the gel/suspended beads with 3x10 mL PBS (*the final 10 mL should be added via a pipette rinsing the sides of the filtration apparatus free of gel/beads*).
10. Very gently remove the filter paper from the filtration apparatus and place in a glass centrifuge tube (*with a spatula try to remove any residual gel from around the filter funnel and apply to the filter paper*).
11. Suspend the gel/beads and filter paper in 2.5 mL PBS.
12. Add 20 µL of the mercaptoethanol solution and 200 L of the OPNG solution , incubate for 15 minutes.
13. Add a 500 µL aliquot of 1 M sodium carbonate buffer and mix.
14. Centrifuge at 2500 rpm for 5 minutes and then pipette the solution into a UV spectrophotometer cuvette leaving the gel matrix at the bottom of the vial (sample solutions will be coloured).
15. The blank should be set at 420 nm and absorbance for all samples at 420 nm should be recorded.

A typical sequence of samples would be:

1. Blank
2. Blood group A control
3. Blood group A sample
4. Blood group B control
5. Blood group B sample

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Results

The absorbance at 420 nm provides a qualitative assessment of the degree of conjugation between the oligosaccharide and the Sepharose gel solution/suspended beads. The absorbance of the sample solutions should be greater than that of the control samples.

Use/Regeneration

These gels will bind anti-A and anti-B antibodies (haemagglutinins) from plasma or plasma derived products. The bound antibodies can also be recovered by subsequently washing the gels with a low pH buffer (such as 0.1 M glycine-HCl pH 2.2). Alternatively the gels may be regenerated by washing with a high salt buffer at neutral pH.

Use the gels only in the pH range of 6.5 to 8.0 to perform immunoadsorption. Performance outside this range cannot be guaranteed.

Do not allow the gels to dry out or become partially dehydrated otherwise full reconstitution cannot be guaranteed.

Fully washed gels can be reused and by careful use under these conditions, our gels have been used repeatedly by customers for several years.

Storage

The gels are supplied in 20% (v/v) ethanol in water.

We recommend long term storage of the products in a bacteriostatic medium such as phosphate buffered saline containing 0.02% sodium azide, 0.05% sodium azide in water or 20% (v/v) ethanol in water at 4 °C. Do NOT freeze.

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