

Integrin Receptor Ligand Binding Assays

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- 1- Coat purified integrins (100ul/well) on Costar high capacity binding plates – on at 4C
- 2- Block for 1h at RT then wash with blocking/binding buffer
- 3- Add 100ul of various dilutions of biotinylated extracellular protein ligand (as controls use fibronectin/vitronectin) in binding buffer and incubate for 3h at RT
- 4- Wash plates 3 times with binding buffer and incubate for 1h at RT with anti-biotin horseradish peroxidase (100ul /well) in binding buffer
- 5- Wash plates twice with binding buffer followed by addition of 100ul of peroxidase substrate
- 6- Measure absorbance at 490nm.

Blocking/Binding buffer:

50mM Tris HCl pH7.4
100mM NaCl
2mM CaCl₂
1mM MgCl₂
1mM MnCl₂
1% BSA