

Blot overlay assay

1. Prepare GST- and MBP- recombinant purified proteins
2. Separate ~1-3 μ g of affinity-purified GST recombinant proteins by SDS-PAGE and transfer on nitrocellulose membrane
3. Pre-wet membrane and wash with TBST x5 times to get rid of SDS
4. Incubate membrane overnight at 4oC in Blocking buffer
5. Wash 3x in 1xTBST
6. Overlay with eluted MBP-recombinant protein (~1-3 μ g protein) in Binding buffer
7. Incubate for 4hrs at RT with gentle rocking to allow for binding to occur
8. Wash the membrane 3x with wash buffer for 5 min at RT, and 1x wash in TBST
9. Incubate with primary antibody (GST antibody) and continue as normal for immunoblotting protocol

Note: Reciprocal reactions can also be performed (ie run MBP-proteins and overlay with GST-eluted protein)

Blocking buffer

50 mM Tris-HCl pH 7.5

150 mM NaCl

0.05% (v/v) Tween-20

2 mM DTT

0.5% NP-40,

5% nonfat milk

Binding buffer

50 mM Tris-HCl pH 7.5

150 mM NaCl

0.05% (v/v) Tween-20

2 mM DTT

0.5% NP-40

2mM ATP

5% nonfat milk

Wash buffer

50 mM Tris-HCl pH 7.5

150 mM NaCl

0.05% (v/v) Tween-20

2 mM DTT

1% NP-40