

GST-Protein and IVT Binding Assay

- 1) Use 15 ug GST-MAGE-B18/C2 (test samples) or 15 ug of GST (neg. control)
- 2) Wash 10 uL of Glutathione-beads in 400uL TBST, repeat (in 0.5mL tubes)
- 3) Add 50 uL of TBST (supplemented with 10 mM B-Me) + GST-tagged proteins of interest (GST alone or GST-MAGE-B18/C2)
- 4) Mix for 1 h in vibrating mixer (level 3-4) at RT
- 5) Spin down and Remove supernatant
- 6) Add 50 uL of TBST + 5% milk + 10 mM B-Me
- 7) Mix for 1 h in vibrating mixer (level 3-4) at RT
- 8) Remove supernatant (beads are hard to see, so be careful)
- 9) Add 50 uL of TBST + 5% milk + 10 mM B-Me
- 10) Add 5 uL in vitro translated protein of interest (myc-LNX1). Keep 1 uL as input.
- 11) Mix for 1 h in vibrating mixer (level 3-4) at RT
- 12) Wash beads (red) 4X with 400uL TBST + 10mM B-Me
- 13) Remove all extra sup with pipette and add 20 ul of 2X SDS-SB and boil 5-10 min
- 14) Also add 20ul 1X SDS-SB to input
- 15) Load 10 uL of input (10%), and 18 uL of bound