**GST-IR purification**

- 2 liters of LB
- Incubate with GST-IR transformed BL21
- Incubate 1 liter with 5X salt. Off culture.
- Grow to 0.5 approx. 0.6 at 600 nm
- Add IPTG 100um and inducing for 4 hours at 37°C
- Pellet cells freeze and store at -20°C if needed
- Resuspend in wash 5X E buffer, pH 7.5

- Add 1.5 ml NeoN at pH 8.0 and resuspend per ml completely. Place on ice for 15 min
- Add 20ul PMSF 100um (boil peptide, Send Apoptosis)
- Incubate for 1 min
- Repeat again. Keep in ice.
- Centrifuge 4°C, 10000 rpm. Collect sup.
- Add 1ml glutathione beads and rotate at 4°C for 2 hours
- pellet beads
- Wash in NeoN 5 times
- Resuspend in 1 ml NeoN

**Load Cells**

1. GST-IR pellet 25 ul Set 1
2. GST-IR sup 25 ul
3. GST-IR beads 10 ul

**2**

1. GST-IR pellet 25 ul Set II
2. GST-IR sup 25 ul
3. GST-IR beads 10 ul