

Immunoprecipitation of cells

- 200 million cells
- Wash in 10 ml PBS
- Lyse in 1 ml NETN 0.5% NP40 30 minutes
Vortex every 10 mins - on ice
- pellet 5 mins 1500 rpm
- Remove 1 ml Sup to 1.5 ml epp.
- Add 0.5 ml NETN 1% NP40 30 minutes
Vortex every 10 mins - on ice
- pellet 1800 rpm 10 mins
- pool Sups. Add 5 μ l NRS 30 mins rotating 4°C
- Add 50 μ l Protein A/G 30 mins rotating 4°C
- 2 mins centrifuge max. Transfer Sup to fresh 1.5 ml epps.
- Add 10 μ l J Kappa serum to sup. Incubate 4°C o/n rotating.
- Add 50 μ l Protein A/G 1 hour rotating 4°C
- Wash 4 x 0.5% NP40, NETN.
- Boil in 50 μ l SDS loading Buffer
- 1% lysate (2 million) } load gels.
NRS
J Kappa 1
J Kappa 2

BL41
BL41/B95
IB4
LCL1

SupT1
Hut78
AKY
Turkat