

Immunoprecipitation Buffer

Prepare pG agarose solution by washing with lysis buffer* and spinning down at 3000 rpm for 2 min (repeat once).

Prepare largest samples possible (all samples should have equal ug) and add lysis buffer so that all samples are equal in volume.

Preincubate samples in pG solution for 1 hour on 4°C agitator.

Spin down for 1 min at 13000 rpm.

Transfer supernatant to new tube.

Repeat preceding 2 steps.

Add Ab and incubate for 1 hour on 4°C agitator.

Add pG solution.

Incubate for 1 hour on 4°C agitator.

Spin for 3 min 3000 rpm at 4°C.

Remove supernatant.

Wash with 500 ul lysis buffer.

Repeat preceding 3 steps (spin, remove supernatant, wash).

Resuspend bead with 30 ul SBS 1 + DTT 100 mM

for 500 ul:

100 ul SBS 5X

50 ul DTT 1M

350 dH₂O

*Lysis Buffer:

10 ml TGEK100

PMSF (1/200)

Benzamidine (1/200)

Leupeptine (1/500)

Aprotinin (1/200)

NP40 1%