

IMMUNOPRECIPITATION from tissue

1. Place the tissue (10 heads / 10 larvae) in 1ml of PLPC lysis buffer:

for 50ml of PLPC

25ml	2X base (see below)	-> 1X (50mM HEPES, 150mM NaCl, 10% glycerol)
100µl	0.5M EGTA (pH 7.5)	-> 1mM
375µl	0.2M MgCl ₂	->1.5mM
5ml	1M NaF	->100mM
5ml	100mM sodium pyrophosphate	->10mM
1.17ml	1mM pepstatin A	->0.01mg/ml
730	mM leupeptin	->0.01mg/ml
500	100mM PMSF	->1mM
50	100mM sodium orthovanadate	->0.1mM
5ml	10% Triton-X	->1%
7.1ml	ddH ₂ O	

for 50ml of 2X base

5ml	1M HEPES (pH 7.5)	-> 100mM
3ml	5M NaCl	-> 300mM
10ml	glycerol	->20%

2. Homogenize the tissue for 2 minutes.

3. Microfuge @ 4°C until the supernatant is clear.

4. Set aside 100 for a control. For pre-clearing, to the remaining 900 , add 50 of rabbit serum and incubate @4°C on the nutator for 1 hour.

5. Now add 100 of Protein A beads (these should be equilibrated with the lysis buffer beforehand) and incubate for 1hr @ 4°C.

6. Spin for 15 seconds at 4°C in the microfuge and remove the supernatant.

7. Meanwhile add 13 of a-DFur2 antibody to 900 of the pre-cleared lysate and rotate on the nutator for 1hr @ 4°C.

8. Then add 90 of Protein A beads (again, these should be pre-equilibrated in lysis buffer before use). Mix for 1 hour @ 4°C.

9. Spin for 15 seconds in the microfuge and remove the supernatant.

10. Wash the beads in 5ml of ice cold HNTG wash buffer 4X 5'.

HNTG

1M HEPES (pH 7.5)	->20mM
5M NaCl	->150mM

10% Triton-X ->0.1%
glycerol ->10%

HNTG Wash Buffer

11. Resuspend the beads in loading buffer. Boil
12. Spin in the microfuge, remove the supernatant to load on the gel.