

## Ovarian immunostaining procedure II

This procedure is adapted from Xu and Ruohola-Baker's approach to staining follicle cells for cut. We have found that though lacZ staining is weaker than the PSN approach, the background is very low so photomicrographs are robust.

Dissect in Ringers + a pinch of BSA

Remove excess ringers and fix in 4% paraformaldehyde in 0.3ml PBS, 15-30 min

Remove excess fix and wash 2X in NPS (0.4ml/wash; see recipe for NPS below)

Remove wash and block (2 hours/overnight) in NPS + 20% BSA (250ml NPS + 50ul BSA)

Remove excess block and wash 2X in NPS

Add primary antisera (200ul) and incubate O/N

Remove excess primary and wash 2X in NPS

Add secondary antisera (200ul; for Alexa dyes, 1:200) and incubate O/N

Remove excess secondary and wash 3X in NPS (if appropriate, 2<sup>nd</sup> wash can contain DAPI at 1:1000)

Wash 1X in 50% glycerol and mount

### NPS

	<u>50ml</u>
50mM Tris-7.4	2.5ml of 1M stock
150mM NaCl	1.5ml of 5M stock
0.5% NP-40	0.25ml (or 5ml of 5% stock)