

## Antibody Staining Early Embryos

This works well on pre-implantation embryos to ~ e7.5; also nuclear staining

1. Fix embryos with 4% PFA in PBS for 10-30 min or o/n at 4C
2. Wash in PBS + 0.1% Triton-X100, 3 times
3. Permiabilize embryos in PBS + 0.25% Triton for 15 min to 30 min at RT
4. Wash in PBS + 0.1% Triton, 3 times
5. Block with + 10% FBS in PBS (without  $\text{Ca}^{2+}/\text{Mg}^{2+}$  and with 0.1% Triton for at least 1hr- at RT)

(if you want to store your embryos, you can do at this step and keep them at 4C)

6. First Ab reaction in blocking solution (10% FBS + PBS + 1% Triton) O/N at 4C (or 1hr at room temp) in a Terasaki plate. (usually 1at /50 to 1/500 dilution)
7. Wash in PBS + 0.1% Triton for 10 min 3 times
8. Second Ab reaction in PBS + 0.1% Triton for 1hr at RT  
Most of our 2<sup>nd</sup> Abs are used at 1/400 dilution
9. Wash in PBS 0.1% Triton 10 min 3 times

(Optional)

10. Mount embryos in Draq5. Start imaging after 15 min incubation at room temperature