antibody staining of fly embryos

required reagents
100% methanol
37% formaldehyde
1X PBS
50% bleach
agar collecting plates
n-heptane
10% goat serum
1X PBT (1X PBS + 0.1% Triton)
distilled water
yeast paste

staining procedure
-collect embryos on embryo collection plates containing small amount of wet yeast paste
-rinse embryos onto wire mesh baskets with distilled water and 000 paint brush
-dechorionate with 50% bleach for 3 minutes
-rinse embryos three times with distilled water
-set up microfuge tubes (one tube per basket)
-add to each tube 450μL 1X PBS, 50μL 37% formaldehyde (mix), 500μL n-heptane
-transfer embryos from basket to microfuge tube
(wet paintbrush in n-heptane layer, scoop up embryos, dip in heptane layer)
-lay microfuge tubes on their sides on table top shaker
-shake vigorously for 20 minutes at room temperature
-allow layers to separate for 1 minute at room temperature
-remove fixative layer (bottom layer), fixed embryos will remain at the interface
-add 500μL 100% methanol
-devitilllize by shaking vigorously for 1 minute at room temperature
-remove entire solution and embryos at the interface
(devitilllized embryos sink to the bottom of the tube)
-wash three times with 100% methanol
-rehydrate and nitate in 50:50 (500μL 100% methanol: 500μL 1X PBT) for 10 minutes at room temp
-rehydrate and nitate in 1000μL 1X PBT for 10 minutes at room temp
-block and nitate in 10% goat serum for 10 minutes at room temp
-gently shake in primary antibody (dilute in 10% goat serum) overnight
-wash and nitate in 1000μL 1X PBT for 10 minutes at room temp
-gently shake in secondary antibody (dilute in 10% goat serum) for 3 hours at room temp
-wash and nitate in 1000μL 1X PBT for 10 minutes at room temp
-hydrate in Vectashield for 5 minutes at room temp
-mount on slides (40μL Vectashield + embryos mixture)