Protocol: Yeast DAPI Stain

DAPI is a fluorescent nucleic acid stain. In this protocol ethanol is used as a fixative.

What is needed:
A yeast culture to stain
Phosphate buffered saline (PBS)
Stock DAPI solution (1mg/ml)
Agarose pads for microscopy and coverslips

1. Centrifuge 1ml of yeast for 1 minute at 6,000 x g.
2. Decant supernatant. Wash in 1ml of PBS. Centrifuge for 1 minute at 6,000 x g.
3. Decant supernatant and resuspend in 1ml PBS.
4. Add 333μl of the washed yeast culture to a new 1.5ml microcentrifuge tube.
5. Add 666μl of 100% ethanol to the yeast culture.
6. Incubate the yeast and ethanol mixture at room temperature for 30 minutes. (You may notice clumping of the cells; this is OK as ethanol causes proteins to congeal or stick together.)
7. While the cells are being fixed, make the DAPI solution by adding 1.25μl of a 1mg/ml DAPI stock solution to 1ml of phosphate buffered saline (PBS). Keep this solution in the dark until it is needed in **Step 10** below.
8. Centrifuge the cells for 1 minute at 2,500 rpm.
9. Decant the supernatant. Wash the cells in 1ml of PBS without DAPI. Centrifuge again for 1 minute at 2,500 rpm.
10. Decant the supernatant. Add 200μl of the DAPI stain and resuspend the cells.
11. Add 2μl (up to 5μl depending on cell density) of cells to a microscope slide with agarose pad. Add a drop of VectaShield (if desired). Place coverslip and seal with nail polish.