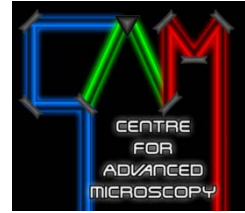




## Centre for Advanced Microscopy General Method

### Antibody Staining of Cells Grown on Coverslips (with optional DPX mounting)



#### Introduction

This method can be used to stain cells that have been grown on coverslips with antibodies or dyes.

#### Materials

- Coverslips – preferably 0.17mm +/- 0.01mm
- Primary and Secondary (if required) antibodies
- Paraformaldehyde (4%) – fresh, no more than two weeks old. Preferably from an ampoule.
- Triton X-100
- PBS
- BSA
- PBT (PBS + 0.1% Triton X-100)
- Block (2% BSA in PBT)
- Ethanol (optional)
- Xylene (optional)
- DPX (optional)

#### Method

1. Grow cells to confluence on coverslips
2. Wash 1x with PBS
3. Fix with 4% paraformaldehyde for 15 minutes
4. Wash 2x with PBS
5. Block cells in PBT+2% BSA for 30 minutes at room temperature
6. Wash 3x with PBT
7. Incubate with Primary antibody for 1-2 hours at room temperature, or overnight at 4°C
8. Wash 2x with PBT
9. Wash 1x with PBS
10. Incubate with Secondary antibody for 1 hour
11. Wash 3x with PBS
12. Mount coverslip to a slide using one of the mounting methods below. Alternatively the coverslip can be imaged directly by placing it in a Sykes-Moore chamber

#### Mounting Options



The coverslip with cells attached can be mounted onto a microscope slide using ProLong Gold (Invitrogen) or VectaShield (Vector Labs). These are glycerol based antifade reagents that will set hard when left at 4°C for 24 hours. For extra protection the edges of the coverslip can be sealed with nail polish.

Alternatively the cells can be mounted using DPX (see method below), a standard hard set histology mountant. This process requires the cells to be dehydrated before mounting. This will result in the cells being very flat, so if cell volume is important this is not an option.

### DPX Mounting Method

- Dehydrate the coverslip in a series of ethanol as follows
  - 70% for 10 minutes
  - 90% for 10 minutes
  - 100% for 5 minutes (do this 3 times)
- Cover coverslip in xylene for 2 minutes
- Drain excess xylene
- Mount using DPX

