

# Staining protocol for unicellular protists: Mitotracker and Dapi Version 2

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## Abstract

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## Protocol

### 2% PFA Fixation

#### Step 1.

Add PFA drop by drop and mixing carefully into 1mL cells for a final concentration of 2%PFA

Incubate cells for 4 min at room temperature

#### Step 2.

Centrifuge at 1000g for 5 minutes

#### Step 3.

Wash cells with 1XPBS twice

#### Step 4.

Resuspend cells with 1mL 1xPBS

#### Step 5.

Add 1mM mitotracker dye

#### Step 6.

Room temperature, protect from light

After 10 minutes, add 1/1000 DAPI dye

#### Step 7.

Room temperature, protect from light

After 5 minutes, wash with 1xPBS twice

#### Step 8.

Centrifuge at 1000g for 5 minutes and resuspend with 1xPBS

Total incubation times:

Mytotracker: 15min

DAPI: 5min

Mount your slide and ready for observation!

**Step 9.**