

# Staining protocol for unicellular protists: LysoTracker and Dapi Version 3

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## Abstract

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## Protocol

### Step 1.

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

### Step 2.

Incubate cells for 4 min at room temperature

⌚ DURATION

00:04:00

### Step 3.

Centrifuge at 1000g for 5 minutes

⌚ DURATION

00:05:00

Wash

### Step 4.

Wash cells with 1XPBS (1/2)

Wash

### Step 5.

Wash cells with 1XPBS (2/2)

Resuspend

### Step 6.

Resuspend cells with 1mL 1xPBS

### Step 7.

Add 1/1000 lysotracker dye: 1 uL of lysotracker for a 1mL final Volume of cells.

Room temperature, protect from light.

### Step 8.

Incubate for 20 min.

Room temperature, protect from light.

 **DURATION**

00:20:00

### Step 9.

Add 1/1000 DAPI dye.

Room temperature, protect from light.

### Step 10.

Incubate for 5 minutes.

Room temperature, protect from light.

 **DURATION**

00:05:00

 **NOTES**

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Total incubation times:

Lysotracker: 25min

DAPI: 5min

Wash

### Step 11.

Wash with 1xPBS (1/2)

Wash

### Step 12.

Wash with 1xPBS (2/2)

### Step 13.

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

 **DURATION**

00:05:00

### Step 14.

Mount your slide and ready for observation!

