

Staining protocol for unicellular protists: Mitotracker and Dapi Version 4

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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)

Step 6.

Resuspend cells with 1mL 1xPBS

Step 7.

Add 1mM mitotracker dye.

Room temperature, protect from light

Step 8.

Incubate for 10 minutes.

Room temperature, protect from light

 DURATION

00:10:00

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

Maria Rubio-Brotons 22 Nov 2016

Total incubation times:

Myotracker: 15min

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!