Staining protocol for unicellular protists: Lysotracker and Dapi Version 2

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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!