

# Triple staining protocol for unicellular protists: LysoTracker, MytoTracker and Dapi

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## Abstract

**Citation:** Maria Rubio-Brotons Triple staining protocol for unicellular protists: LysoTracker, MytoTracker and Dapi. **protocols.io**

[dx.doi.org/10.17504/protocols.io.geubtew](https://dx.doi.org/10.17504/protocols.io.geubtew)

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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 PBS

#### Step 5.

Add 1/1000 lysoTracker dye

#### Step 6.

1 uL of lysoTracker for a 1mL final Volume of cells

Add 1 mM Mitotracker dye after 10 minutes

#### Step 7.

Add 1/1000 DAPI dye after 10 minutes

#### Step 8.

After 5 minutes, wash with 1xPBS twice

#### Step 9.

## ■ ANNOTATIONS

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Total times:

LysoTracker: 25min

Mytotracker: 15min

DAPI: 5min

Mount your slide and ready for observation!

**Step 10.**