

## 13 DNA Content Analysis of Fixed Cells with Propidium Iodide UREAD

SopID: 13 - UREAD

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Transcriptomics

DNA analysis, fixed cells, Propidium Iodide, flow cytometry

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### Backgrounds

--to be edited--

### Overview

This protocol uses ethanol to fix and permeabilise cells, aiding access of dye to DNA in intact cells and allowing DNA content analysis of stained cells by flow cytometry. The use of a fixation step makes this protocol applicable in situations when samples have to be stored before the analysis.

The fixed cells are rinsed with PBS and then stained with the DNA fluorochrome PI in a solution containing Triton X-100 as well as RNase A. Flow cytometry requires excitation with blue light and detection of PI emission at red wavelengths. Alternatively, DAPI (requires UV excitation) can be used instead of PI.

### Materials

Amount	Name	Supplier	Catalogue No.	Further information
	70% ethanol			To fix cells (4.5 ml)
	PBS			To suspend cells
1 ml	PI/Triton X-100 staining solution with RNase A			To suspend cell pellet
	12 x 75 mm centrifuge tubes			Preferably polypropylene or silanized.
	Beckman TJ rotor (or equivalent)			To centrifuge cells
	Pasteur pipette			
	Flow cytometer			
10 ml 2 mg 200 ul	<b>PI/Triton X-100 staining solution with RNase A</b> 0.1% (v/v) Triton X-100 in PBS DNase-free RNase A 1 mg/ml PI	Sigma Sigma Molecular		For ethanol-fixed cells

		Probes		
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## Main Procedures

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- Fix cells with ethanol
- Stain cells with PI
- Perform flow cytometry

## Sub Procedures

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### *Fix cells with ethanol*

#### 1) Fill centrifuge tubes with ethanol

Prepare the fixative by filling 12 x 75 mm-centrifuge tubes with 4.5 ml of 70% ethanol. Keep tubes on ice.

#### 2) Suspend cell in PBS

Collect cells and suspend  $10^6$  to  $10^7$  cells in 5 ml PBS in a centrifuge tube.

#### 3) Centrifuge - 5 minutes

Centrifuge cells 5 min at  $\sim 200 \times g$  (e.g., 1000 rpm in Beckman TJ rotor) and remove supernatant.

#### 4) Resuspend cells

Using a Pasteur pipette thoroughly resuspend cells in 0.5 ml PBS.

*(See additional information, no. 1).*

#### 5) Transfer to tubes containing ethanol - 2 hours

Transfer the cell suspension into the tubes containing 70% ethanol. Keep cells in fixative  $\geq 2$  hr.

*(See additional information, no. 2).*

### *Stain cells with PI*

#### 6) Centrifuge - 5 minutes

Centrifuge the ethanol-suspended cells 5 min at  $200 \times g$ . Decant ethanol thoroughly.

#### 7) Suspend in PBS and centrifuge - 5 minutes

Suspend the cell pellet in 5 ml PBS, wait 60 sec, and centrifuge 5 min at  $200 \times g$ .

#### 8) Suspend in PI - 15 – 30 minutes

Suspend cell pellet in 1 ml PI/Triton X-100 staining solution with RNase A (*see additional information no. 3*). Keep either 15 min at  $37^\circ\text{C}$  or 30 min at room temperature.

### *Perform flow cytometry*

#### 9) Set up flow cytometer

Set up and adjust the flow cytometer for excitation with blue light and detection of PI emission at red wavelengths.

*(See additional information, no. 4).*

#### 10) Measure cell fluorescence in the flow cytometer.

## Safety

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**Users must comply with COSHH and local safety regulations.**

## Additional Information

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1. It is important to achieve a single-cell suspension. Fixation of cells that are in aggregates while suspended in PBS stabilises the aggregates, which are then impossible to disperse. It is essential,

therefore, to have a monodisperse cell suspension at the time of mixing cells with ethanol.

2. Cells suspended in 70% ethanol can be stored at 0° to - 40°C for several months if not years.

3. PI/Triton X-100 staining solution with RNase A, for ethanol-fixed cells - To 10 ml of 0.1% (v/v) Triton X-100 in PBS add 2 mg DNase-free RNase A and 200 ul of 1 mg/ml PI. Prepare freshly. A stock solution of PI, made by dissolving 1 mg PI in 1 ml water, can be stored for several months at 0° to 4°C. If the RNase is not DNase-free, boil a solution of 2 mg RNase A in 1 ml water for 5 min.

4. For excitation, the 488-nm argon ion laser line may be used. Alternatively, use a BG 12 optical filter when the source of illumination is mercury arc or xenon lamp. A long-pass (>600 nm) filter is recommended for detecting PI emission.

### **Definitions**

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PBS - Phosphate Buffered saline

PI – Propidium Iodide