

## **Cell viability**

## How it works

In flow cytometry your data is collected on a per cell basis. So you see information for each cell. This means that a viability dye is a useful addition to exclude dead cells from your data, and when setting up cell sorts.

Viability dyes generally bind to DNA, therefore measure membrane permeability, which is indicative of cell death. However, annexin- V binds to a membrane protein whose abundance increases during apoptosis. Thus, cells which stain positive are dead/undergoing apoptosis.

Reagent list				
Annexin V		Protein	Various suppliers	
PI	Propidium iodide	DNA	Various	
7-AAD	7-amino-actinomycin D	DNA	Various	
Hoechst 33342		DNA	Various	
FluoroGold		DNA	Molecular probes	
LIVE/DEAD viability dyes		Protein	ThermoFisher	

Quick info					
	Laser	Fixable	Slap it in	Starting point	
Annexin V-conjugate	Various	-	No*	Determine	
PI	488, 561 nm	-	Yes	2 μg / 10 <sup>6</sup> cells	
7-AAD	488, 561 nm	-	Yes	10μg / mL	
Hoechst 33342	355 nm	Yes	Yes	10μg / mL	
FluoroGold	355, 405 nm	-	Yes	10μg / 200 μL	
LIVE/DEAD viability dyes	Various	Yes	No*	See kit manual	
*kit available					

## **Protocols**

PI	7-AAD	Hoechst	FluoroGold
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- Harvest cells in single suspension (10<sup>6</sup> cells)
- Wash cells
- Resuspend in stain buffer
- Add stain
- Acquire after 5-10min incubation

Annexin-V	LIVE/DEAD
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See manufacturers' instructions

Annexin-V: Thermo | Merck | abcam

LIVE/DEAD